Evolution in the Genus Rhinella: A Total Evidence Phylogenetic Analysis of Neotropical True Toads (Anura: Bufonidae)

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ABSTRACT

True toads of the genus *Rhinella* are among the most common and diverse group of Neotropical anurans. These toads are widely distributed throughout South America, inhabiting a great diversity of environments and ecoregions. Currently, however, the genus is defined solely on the basis of molecular characters, and it lacks a proper diagnosis. Although some phenetic species groups have traditionally been recognized within *Rhinella*, the monophyly of some of them have been rejected in previous phylogenetic analyses, and many species remain unassigned to these poorly defined groups. Additionally, the identity and taxonomy of several species are problematic and hinder the specific recognition and description of undescribed taxa. In this work, we first perform phylogenetic analyses of separate mitochondrial and nuclear datasets to test the possible occurrence of hybridization and/or genetic introgression in the genus. The comparative analysis of both datasets revealed unidirectional mitochondrial introgressions of an unknown parental species into *R. horribilis* ("ghost introgression") and of *R. dorbignyi* into *R. bernardoi*; therefore, the mitochondrial and nuclear datasets of these species were considered separately in subsequent analyses. We performed total-evidence phylogenetic analyses that included revised molecular (four mitochondrial and five nuclear genes) and phenotypic (90 characters) datasets for 83 nominal species of *Rhinella*, plus several undescribed and problematic species and multiple outgroups. Results demonstrate that *Rhinella* was nonmonophyletic due to the position of *R. ceratophrys*, which was recovered as the sister taxon of *Rhaebo nasicus* with strong support. Among our outgroups, the strongly supported *Anaxyrus* + *Incilius* is the sister clade of all other species of *Rhinella*. Once *R. ceratophrys* is excluded, the genus *Rhinella* is monophyletic, well supported, and composed of two major clades. One of these is moderately supported and includes species of the former *R. spinulosa* Group (including *R. gallardoi*); the monophyletic *R. granulosa*, *R. crucifer*, and *R. marina* Groups; and a clade composed of the mitochondrial sequences of *R. horribilis*. The other major clade is strongly supported and composed of all the species from the non-monophyletic *R. veraguensis* and *R. margaritifera* Groups, the former *R. acrolopha* Group, and *R. sternosignata*. Consistent with these results, we define eight species groups of *Rhinella* that are mostly diagnosed by phenotypic synapomorphies in addition to a combination of morphological character states. *Rhinella sternosignata* is the only species that remains unassigned to any group. We also synonymize nine species, treat three former subspecies as full species, and suggest that 15 lineages represent putative undescribed species. Lastly, we discuss the apparently frequent occurrence of hybridization, deep mitochondrial divergence, and "ghost introgression"; the incomplete phenotypic evidence (including putative character systems that could be used for future phylogenetic analyses); and the validity of the known fossil record of *Rhinella* as a source of calibration points for divergence dating analyses.

INTRODUCTION

General Overview

True toads of the former genus *Bufo* are a popular group of anurans distributed nearly worldwide, and widely studied by researchers from different disciplines. The classic book "Evolution in the genus *Bufo*" (Blair, 1972) synthesized knowledge about the morphology, phylogeny, and biology of the group. Despite having integrated evidence from many sources of characters to elucidate the evolutionary relationships among the species groups of true toads, this work largely revealed the difficulties to study their phylogenetic relationships. It was not until the 1990s–2000s that a general picture of these relationships emerged, and the taxonomy of true toads was revised to be consistent with phylogenetic hypotheses (Graybeal, 1997; Pauly et al., 2004; Frost et al., 2006; Pramuk, 2006). Currently, most of the South American true toads of the former genus *Bufo* are grouped in the large genus *Rhinella* (Chaparro et al., 2007).

Rhinella includes many of the most conspicuous and ubiquitous species of the anuran fauna in almost all the major biogeographic areas of the Neotropical region (Duellman, 1999; Frost, 2020; IUCN, 2020). With 92 species, *Rhinella* is the second largest genus of Bufonidae, and its species show considerable morphological and biological diversity, including large variation in size, different levels of cranial ossification, integumentary structure, larval morphology, and ecological and reproductive diversity characteristics (Trueb, 1971; Cei, 1972a; Toledo and Jared, 1993; Pramuk, 2006; Aguayo et al., 2009; van Bocxlaer et al., 2010; Pereyra et al., 2015; Bandeira et al., 2016; Simon et al., 2016; Hudson et al., 2018). Some common species of *Rhinella* (e.g., *R. arenarum, R. horribilis*, and *R. marina*) have been employed extensively as model organisms for various biological disciplines, such as biochemistry (e.g., Abel and Macht, 1912; Cei et al., 1968; Rash et al., 2011), developmental biology (e.g., Markovich and Regeer, 1999; Barisone et al., 2002; Brown et al., 2002), ecotoxicology (e.g., Lajmanovich et al., 2011), molecular biology (e.g., Estoup et al., 2004, 2010; Rollins et al., 2015; Edwards et al., 2018; Ceschin et al., 2020), and especially physiology (e.g., Houssay and Giusti, 1929; Houssay, 1949; Penhos et al., 1967; Sassone et al., 2015). This genus also contains a highly invasive species, *R. marina*, widely introduced into many countries and islands from different continents (Frost, 2020), where usually it has a highly negative ecological and socioeconomic impact (Jolly et al., 2015; Bacher et al., 2018).

SYSTEMATICS OF *RHINELLA*

For decades, all South American true toads were part of the formerly large and poorly defined genus *Bufo*, which included a heterogeneous group of toads distributed throughout Africa, America, and Eurasia (e.g., Blair, 1972; Graybeal, 1997). Frost et al. (2006) partitioned this polyphyletic genus into monophyletic units mostly on the basis of the results of their phylogenetic analysis but also on the results of previous studies (e.g., Graybeal, 1997; Pauly et al., 2004). Frost et al. (2006) resurrected *Rhinella* for the species of the former *Bufo margaritifer* Group, which they recovered as distantly related to the other species of South American true toads included in their analysis, including *Chaunus* and *Rhaebo* (both also resurrected by Frost et al., 2006). Frost et al. (2006) noted that *Bufo margaritifer* was nested within *Chaunus* in a previous phylogenetic study (Pauly et al., 2004), a finding that was subsequently supported by Pramuk (2006) and Chaparro et al. (2007). Therefore, *Rhinella* was later redefined to include the species of *Chaunus* and *Rhamphophryne* as well (Chaparro et al., 2007).

The species groups of the former *Bufo* now referred to *Rhinella* were all recognized primarily on the basis of osteological characters and external morphology that were interpreted without quantitative phylogenetic analyses (Tihen, 1962; Cei, 1972a; R.F. Martin, 1972a, 1972b; Duellman and Schulte, 1992), including the *R. crucifer, R. granulosa, R. margaritifera, R. marina, R. spinulosa*, and *R. veraguensis* Groups. Pramuk (2006) studied the phylogenetic relationships of these toads on the basis of a combined analysis of morphological (mostly osteological) and molecular evidence. She rejected the monophyly of some of these species groups (e.g., the *R. veraguensis* Group is polyphyletic with respect to *R. ocellata,* the *R. margaritifera* Group, and *Rhamphophryne*), but did not modify their composition or diagnosis.

The subsequent increase in the knowledge of relations within *Rhinella* was limited to the addition of available sequences of some species in extensive phylogenetic analyses of Bufonidae or Anura (e.g., van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Jetz and Pyron, 2018). Figure 1 summarizes the main results of the more inclusive analyses of *Rhinella*.

For well over a decade, the systematics of *Rhinella* as a whole has languished, although several efforts focusing on the relationships and taxonomy of parts of the genus have been undertaken. These include phylogenetic analyses of presumptively monophyletic species groups (i.e., the *R. crucifer, R. granulosa,* and *R. marina* Groups; Maciel et al., 2006, 2010; Thomé et al., 2010, 2012;

Vallinoto et al., 2010; Pereyra et al., 2016a) or fractions of the diversity of certain groups (i.e., the *R. festae* and *R. margaritifera* Groups; Fouquet et al., 2007a; Moravec et al., 2014; Santos et al., 2015; Cusi et al., 2017; Avila et al., 2018). Most recent studies on *Rhinella* aimed primarily to resolve species-level taxonomic problems (e.g., Fouquet et al., 2007a; Narvaes and Rodrigues, 2009; Jansen et al., 2011; Grant and Bolívar-G., 2014; Moravec et al., 2014; Cusi et al., 2017). Consequently, more than a decade after Pramuk's (2006) revision, species groups remain poorly defined, several species cannot be assigned to any of them, and few additional phenotypic synapomorphies have been proposed for *Rhinella* or its internal clades (Hoogmoed, 1986; 1990; La Marca and Mijares-Urrutia, 1996; Pramuk, 2006; Chaparro et al., 2007; Padial et al., 2009; Blotto et al., 2014; Grant and Bolívar-G., 2014; Pereyra et al., 2016a).

Natural hybridization is common in several groups of Bufonidae, including many species of *Rhinella* (Blair, 1972; Feder, 1979; Haddad et al., 1990; Masta et al., 2002; Azevedo et al., 2003; Green and Parent, 2003; Yamazaki et al., 2008; Fontenot et al., 2011; Guerra et al., 2011), and mitochondrial and nuclear introgression have been corroborated in some of these clades (e.g. Green and Parent, 2003; Yamazaki et al., 2008; Fontenot et al., 2011; Dufresnes et al., 2019). Pereyra et al. (2016a) demonstrated the occurrence of hybridization events in the *R. granulosa* Group and unidirectional mitochondrial introgression of *R. dorbignyi* into *R. bernardoi*. A similar situation might exist between *R. marina* and *R. diptycha*, although the evidence is not conclusive (Sequeira et al., 2011; Vallinoto et al., 2017). The impact of these phenomena on the inference of phylogenetic relationships (Hennig, 1966; McDade, 1992; Posada and Crandall, 2002) could be mitigated, at least partially, if detected. A detailed evaluation of the discordance between mitochondrial and nuclear genomes together with a critical taxonomic evaluation provide an effective way to detect hybridization/introgression (Pereyra et al., 2016a).

In this paper, we present a densely sampled phylogenetic analysis of *Rhinella*, including 83 of its 92 species, using molecular (four mitochondrial and five nuclear genes) and phenotypic characters (90 characters from multiple character systems). The goals of this study are to (1) perform a stringent test of the monophyly of *Rhinella* as well as similar tests on all its species groups, (2) identify phenotypic synapomorphies to diagnose the species groups of *Rhinella*, and (3) to evaluate the taxonomic status of several taxa.

MATERIAL AND METHODS

Taxonomic Sampling

For the complete dataset (molecular and phenotypic), we sampled 83 described species of *Rhinella* (including all but nine of the currently recognized species), and 36 exemplar species of other bufonid genera as outgroups (see below). The outgroup species were chosen to provide a severe test of the monophyly of *Rhinella*, whereas the dense sampling within *Rhinella* allowed us to rigorously test the monophyly of all its species groups. All specimens scored for phenotypic data were associated with the most morphologically similar and/or geographically closest conspecific terminal of the molecular dataset for the total evidence (TE) analysis.

Collection and locality data of vouchers for sequences used in this study, including the information of the sources of the sequences (this work or previous studies), are detailed in appendix 1, and GenBank accession numbers are listed in appendix 2. A list of the species, specimens, and bibliography analyzed for character scoring of the phenotypic dataset is given in appendix 3, and the collection and locality data of specimens studied for morphology are provided in appendix 4.

Outgroups

For outgroup sampling, we considered the results of the most recent phylogenetic analyses (Frost et al., 2006; Pramuk, 2006; van Bocxlaer et

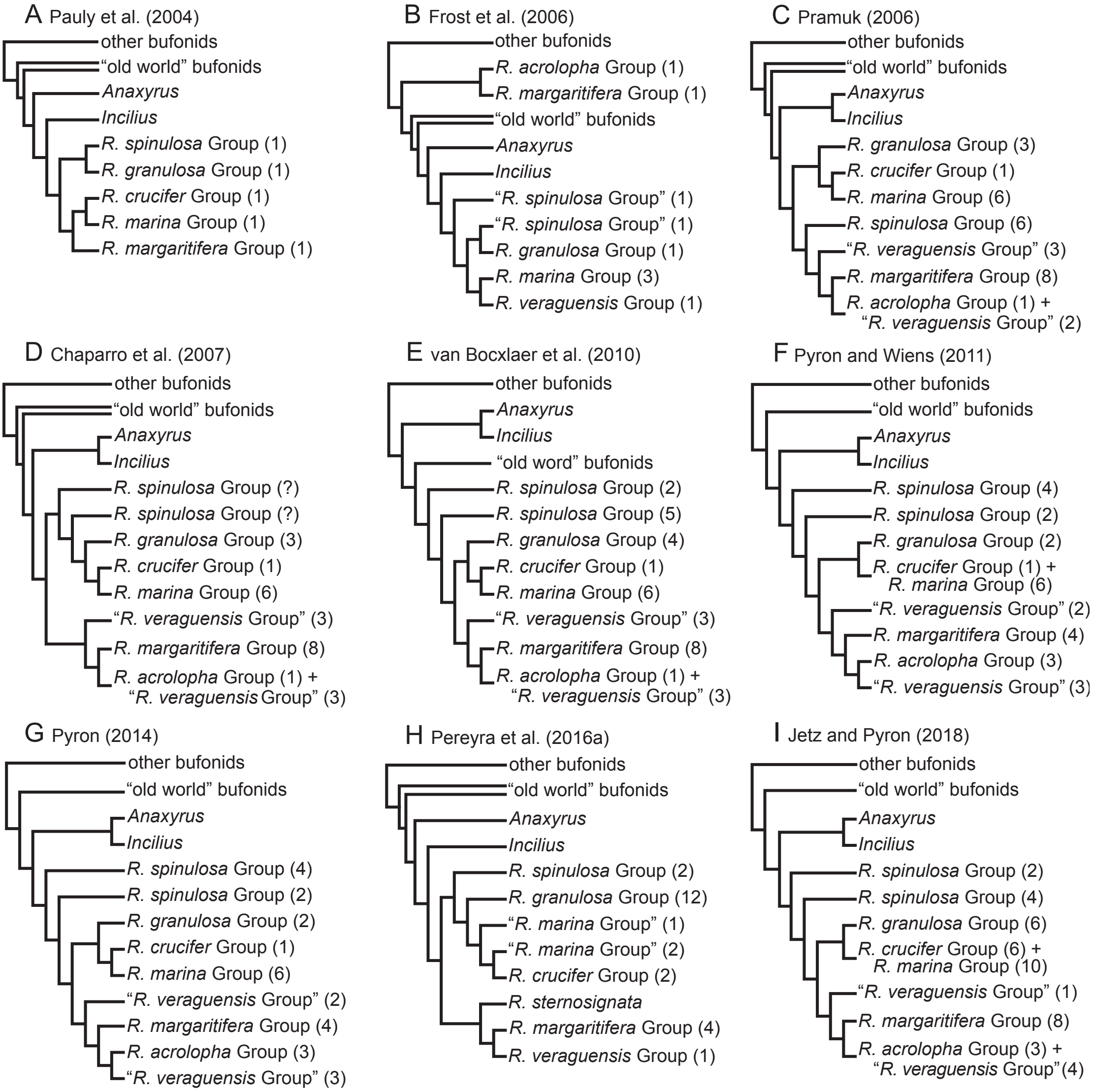

FIG. 1. Summarized relationships of *Rhinella* according to the main published phylogenetic hypotheses of the group. Only the topological sections corresponding to *Rhinella*, and putative most related outgroups (i.e., *Anaxyrus* and *Incilius*) are shown. The number of species sampled within each clade is reported in parentheses. (A) Pauly et al. (2004: fig. 2). (B) Frost et al. (2006: fig. 50). (C) Pramuk (2006: fig. 4). (D) Chaparro et al. (2007: fig. 9). (E) van Bocxlaer et al. (2010: fig. S1). (F) Pyron and Wiens (2011: fig. 2). (G) Pyron (2014: suppl. information "amph_shl.tre"). (H) Pereyra et al. (2016a: fig. 3 and appendix S12). (I) Jetz and Pyron (2018: suppl. information "amph_shl_new.tre").

al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Portik and Papenfuss, 2015; Jetz and Pyron, 2018). The previous hypotheses disagree about the phylogenetic placement of *Rhinella*, recovering it: (1) as closely related to *Incilius* and *Anaxyrus*, and deeply nested within an "old world" bufonid clade (Pauly et al., 2004; Frost et al., 2006; Pramuk, 2006; Chaparro et al., 2007; Pereyra et al., 2016a); (2) as sister taxon of a clade containing all the "old world" bufonid genera (van Bocxlaer et al., 2010); or (3) in a clade together with *Anaxyrus* + *Incilius* that is, in turn, sister taxon of the "old world" bufonid clade (Pyron and Wiens, 2011; Pyron, 2014; Portik and Papenfuss, 2015; Ron et al., 2015; Jetz and Pyron, 2018). As all alternative hypotheses have low support values for most relevant nodes around *Rhinella*, we preferred to sample a broad diversity of bufonid genera representing most of the phylogenetic diversity of the family to rigorously test the relationships and monophyly of the genus. Consequently, we targeted 36 species of 22 bufonid genera as outgroup taxa for the combined molecular dataset and 21 of these species for the phenotypic dataset. Outgroup sequences were obtained exclusively from GenBank (see appendices 1, 2). Thus, in order to increase the number of included genes for outgroup terminals (considering that the number of sampled genes for the ingroup in this work was higher than previous phylogenetic analyses of Bufonidae), we combined sequences from different specimens of the same species to construct several composite outgroup terminals (see justification by Campbell and Lapointe, 2009). These composite terminals (see appendices 1, 2) were constructed only when their uncorrected p-distances (UPDs) in the 16S rRNA gene were less than 0.5%, which is less than the estimated mean divergence observed between sister species of most anurans (Vences et al., 2005a; Fouquet et al., 2007b; Funk et al., 2011). In taxonomy, the exclusive use of pairwise distances and fixed thresholds is questionable (e.g., Will and Rubinoff, 2004; Grant et al., 2006; Meier et al., 2008), but they serve as a useful heuristic for species identification and, in the present context, reduce the risk of constructing composited terminals that could compromise the phylogenetic analysis. Moreover, preliminary analyses including all the sequences of both conspecific specimens recovered them as monophyletic with high support (parsimony jackknife supports >97%, see below).

The Ingroup: *Rhinella*

We included 278 terminals representing 83 described species of *Rhinella* for the combined (molecular + phenotypic) dataset. For practical purposes, the included taxa are presented below in the species groups to which they were assigned by Duellman and Schulte (1992), but considering subsequent modifications to this proposal (details of the assignation of each species to species groups by different authors are given in appendix 5).

For the purposes of our analysis, we recognize the following seven species groups within *Rhinella*: the *R. acrolopha* Group, the *R. crucifer* Group, the *R. granulosa* Group, the *R. margaritifera* Group, the *R. marina* Group, the *R. spinulosa* Group, and the demonstrably paraphyletic "*R. veraguensis* Group." Moravec et al. (2014) also proposed the *Rhinella festae* Group to include three species of the former *Rhamphophryne* and four species of the paraphyletic *R. veraguensis* Group (see Pramuk, 2006; Chaparro et al., 2007; van Bocxlaer et al., 2010; Pyron and Wiens, 2011), which they recovered as a clade in their molecular phylogenetic analysis. Although this resolves the nonmonophyly of the analyzed species of the *R. veraguensis* Group, the authors did not diagnose either their *R. festae* Group or their restricted *R. veraguensis* Group or address the placement of the remaining species of the former *Rhamphophryne*. Given that recognizing the *R. festae* Group left many species of the former *Rhamphophryne* and *R. veraguensis* Group s.l. unassigned to any group due to the lack of diagnoses, we exclude the *R. festae* Group below.

Grant and Bolívar-G. (2014) proposed the *Rhinella acrolopha* Group to include the species previously assigned to *Rhamphophryne*. Although molecular phylogenetic analyses have consistently supported the monophyly of this group (albeit on the basis of a small fraction of its species; van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Jetz and Pyron, 2018), its recognition renders the *R. veraguensis* Group paraphyletic (see Pramuk, 2006; Chaparro et al., 2007; van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Jetz and Pyron, 2018). Thus, as discussed by Grant and Bolívar-G. (2014), the composition and diagnosis of the *R. festae* Group, the *R. acrolopha* Group, and the *R. veraguensis* Group are problematic and will be addressed in the context of our results. For the time being, we employ the putatively monophyletic *R. acrolopha* Group and demonstrably paraphyletic "*R. veraguensis* Group" only to characterize and provide background on the ingroup.

The *Rhinella acrolopha* Group

This group consists of 10 small to medium-sized species of *Rhinella* characterized by a projecting snout, small and inconspicuous parotoid macroglands, heavily ossified skull with some degree of co-osification, well-defined cranial crests (at least in some species), tympanic membrane and annulus absent (except in *R. truebae*), m. levator mandibulae externus undivided with trigeminal nerve passing medial (deep) to the muscle, m. adductor longus absent, and large and unpigmented eggs (Trueb, 1971; Lynch and Renjifo, 1990; Grant and Bolívar-G., 2014). These species are distributed from southern Panama to southern Ecuador, and many of them are critically endangered (Rueda-Almonacid et al., 2004).

We sampled the following species: *Rhinella acrolopha, R. festae, R. lindae, R. macrorhina, R. nicefori, R. paraguas, R. ruizi*, and *R. tenrec*. We also included an undescribed species from Colombia (*Rhinella* sp. C sensu Machado et al., 2016). Sequences of *R. macrorhina* and *R. rostrata* available from GenBank (A. G. Gluesenkamp, unpublished) were not included because our preliminary analyses (data not shown) revealed that the sequences of the fragments of 12S and 16S rRNA genes of each specimen appear to be chimeric and/or contaminated with *R. festae*, and we cannot determine with certainty which sequences correspond to each taxon (see also Cusi et al., 2017). Tissues samples of *R. rostrata* were not available for this study. This poorly known species (Noble, 1920) was described from "Santa Rita Creek," 23 km N of Mesopotamia town, in the southern part of the departamento de Antioquia, Colombia. There is great uncertainty about this locality, because it has never been possible to locate or document it in the literature a stream with that name near Mesopotomia (today part of the municipality of La Unión, Antioquia). Additionally, we could not obtain samples of *R. truebae*, a species known only from the holotype and for which the precise locality is unknown (Lynch and Renjifo, 1990; Vélez-Rodríguez, 2004a).

The *Rhinella crucifer* Group

This putatively monophyletic species group is currently composed of six medium-sized species whose distribution is mainly associated with the Atlantic Forest of Argentina, Brazil, and Paraguay (Duellman and Schulte, 1992; Baldissera et al., 2004; Thomé et al., 2010, 2012; Roberto et al., 2014). The following characters have been proposed to diagnose this species group: skull heavily ossified with slightly elevated cranial crests, dorsal skin smooth with low, scattered tubercles, lateral row of enlarged tubercles present, pale mid vertebral line well-defined, and parotoid macroglands elongated, moderate in size (Duellman and Schulte, 1992; Baldissera et al., 2004; Pramuk, 2006). This species group was recognized as distinct from the *Rhinella marina* Group by R.F. Martin (1972b) and Duellman and Schulte (1992) and all its forms were considered as a single species (*Bufo crucifer*) for a long time (see Lutz, 1934; Cochran, 1955; Cei, 1980; Duellman and Schulte, 1992).

Baldissera et al. (2004) revised the taxonomy of this species group and recognized five species based on morphology and morphometrics: *Rhinella abei* (Baldissera et al., 2004), *R. crucifer* (Wied, 1821), *R. henseli* (Lutz, 1934), *R. ornata* (Spix, 1824), and *R. pombali* (Baldissera et al., 2004). Subsequent to the revision of Baldissera et al. (2004), two additional species, *Rhinella inopina* and *R. casconi*, were described from wet forests within the Cerrado and Caatinga habitats of Brazil, respectively (Vaz-Silva et al., 2012; Roberto et al., 2014). Pramuk (2006) only included one species (*R. ornata,* as *Bufo crucifer*) of this group in her phylogenetic analysis, and recovered it as the sister taxon of the *R. marina* Group. Thomé et al. (2010, 2012) corroborated the monophyly of the *R. crucifer* Group although the outgroup sampling was limited. They also highlighted problems in the taxonomy proposed by Baldissera et al. (2004), as the recognized species did not fully correspond with genetic structuring in the group. Thomé et al. (2010, 2012) found that samples from specimens identified as *R. pombali* are nested within *R. crucifer* and/or *R. ornata* in the mitochondrial phylogenies and are associated with intermediate nuclear genomes in nonphylogenetic analysis (see factorial correspondence analyses [FCA] in Thomé et al., 2012). In addition to these results, a geographic distribution between that of *R. crucifer* and *R. ornata* (Baldissera et al., 2004) is congruent with *R. pombali* as a hybrid complex between the last two species (Thomé et al., 2010, 2012). Furthermore, samples from *R. abei* were nested within *R. ornata*. Thomé et al. (2012) proposed to synonymize *R. pombali* with both parental species and suggested further reassessment of the taxonomic status of *R. abei* with additional molecular markers. Their results were congruent with 2D geometric morphometrics of the skull performed by Bandeira et al. (2016), who found *R. pombali* to be morphologically intermediate between *R. crucifer* and *R. ornata*, and *R. abei* nested within *R. ornata* in the multivariate space.

Several specimens of the six valid species (*Rhinella abei, R. casconi, R. crucifer, R. henseli, R. inopina*, and *R. ornata*) were included in our analyses to test the monophyly of this group and the results of Thomé et al. (2010, 2012). We carried out a preliminary analysis (data not shown) including additional nuclear and mitochondrial sequences of two specimens of "*R. pombali*" and the results supported their findings (see Hybridization and genetic introgression in *Rhinella* section), so we did not include specimens of "*R. pombali*" in our subsequent analyses.

The *Rhinella granulosa* Group

This monophyletic species group is currently composed of 14 medium- to small-sized species of *Rhinella* (Pramuk, 2006; Pereyra et al., 2016a; Murphy et al., 2017). The following characters have been proposed to diagnose this species group: skull heavily ossified and exostosed with low, granular or elevated cranial crests, dorsal skin with small, keratinous-tipped tubercles, and lateral row of enlarged tubercles absent (Gallardo, 1957, 1965; R.F. Martin, 1972a, 1972b; Cei, 1980; Duellman and Schulte, 1992; Pramuk, 2006). All species of the *R. granulosa* Group are mostly distributed in open areas of South America and Panama (Gallardo, 1965; Duellman and Schulte, 1992; Duellman, 1999; Narvaes and Rodrigues, 2009; Sanabria et al., 2010).

The taxonomy of this species group was first revised by Gallardo (1965) and more recently by Narvaes and Rodrigues (2009). The latter authors recognized and diagnosed 12 species on the basis of morphological and morphometrical analyses. Subsequently, Sanabria et al. (2010) described a new species (*R. bernardoi*) from San Juan, western Argentina. The phylogenetic analyses of Pramuk (2006) and Pereyra et al. (2016a), comprising very different samples of species and characters, recovered this species group as monophyletic and discussed several of its phenotypic synapomorphies. Moreover, Pereyra et al. (2016a) documented the occurrence of hybridization between sympatric species as well as past mitochondrial introgression and proposed several morphological synapomorphies for the group. Vera Candioti et al. (2016) proposed some additional synapomorphies from the embryonic morphology (a very short third pair of gills, type A adhesive glands, the adhesive gland subdivision immediately before the gills reach their maximum development, and a short dorsal line of hatching glands mostly restricted to the cephalic region). More recently, Murphy et al. (2017) found the populations of *R. humboldti* on both sides of the Andes to be phylogenetically distinct, leading them to restrict *R. humboldti* to the western Andean populations and resurrect *R. bebeei* for the eastern ones.

In our phylogenetic analyses, we included most species of this group (*Rhinella azarai, R. beebei, R. bergi, R. bernardoi, R. centralis, R. dorbignyi, R. fernandezae, R. granulosa, R. humboldti, R. major, R. merianae, R. mirandaribeiroi,* and *R. pygmaea*) with the exception of *R. nattereri*, a species known from a restricted area in the border between Brazil, Guyana, and Venezuela (Bokermann, 1967; Narvaes and Rodrigues, 2009).

The *Rhinella margaritifera* Group

The definition of this species group is controversial, as diagnoses have been largely based on morphological variation of the *Rhinella margaritifera* species complex (e.g., R.F. Martin, 1972b; Hoogmoed, 1986; Pramuk, 2006) or subjective notions of similarity without consideration of character polarity (e.g., Cei, 1972a; Hoogmoed, 1990; Duellman and Schulte, 1992). The following characters have been used to diagnose this species group: skull relatively lightly ossified with variable amounts of dermal ornamentation and prominent cranial crests, dorsal skin smooth or with small, scattered tubercles, and a lateral row of enlarged tubercles present (Hoogmoed, 1990; Duellman and Schulte, 1992; Vélez-Rodríguez, 2004b; Pramuk, 2006). Nevertheless, this definition does not accomodate the morphology of species recently included in the group (*R. ocellata* and *R. yunga*, the putative sister species to the remaining species of the group, see Moravec et al., 2014).

Similarly, the taxonomy of the species of the *Rhinella margaritifera* Group is also conflicted due to imprecise type localities, extreme sexual dimorphism, and the extensive ontogenetic variation that hinder the specific recognition and description of some putative undescribed species (Hoogmoed, 1977; 1986; 1990; Hass et al., 1995; De la Riva et al., 2000; Vélez-Rodríguez, 2004b; Fouquet et al., 2007a, 2007b, 2007c; Lavilla et al., 2013, 2017). Currently, this group is composed of 20 medium-sized species (see appendix 5) distributed from Panama to southern Brazil, including the Amazonia and Guiana Shield.

We sampled 17 species of this group: *Rhinella acutirostris, R. alata, R. castaneotica, R. dapsilis, R. gildae, R. hoogmoedi, R. lescurei, R. magnussoni, R. margaritifera, R. martyi, R. ocellata, R. paraguayensis, R. proboscidea, R. scitula, R. sclerocephala, R. stanlaii,* and *R. yunga.* This sampling also includes numerous specimens of the *R. margaritifera* species complex throughout its distribution. Additionally, we included two undescribed species of this group, one from Ecuador and another one from Colombia, Peru, and Venezuela. Two species of this group were unsampled: *R. roqueana,* which occurs along the lowlands east of the Andes in southern Ecuador and adjacent northern Peru (Hoogmoed, 1990), and *R. sebbeni*, which is known only from a few localities of the riparian and dry seasonal forests in the Cerrado biome (Vaz-Silva et al., 2015).

The *Rhinella marina* Group

This species group is currently composed of 11 large species (Duellman and Schulte, 1992; Maciel et al., 2010; Vallinoto et al., 2010; Lavilla and Brusquetti, 2018). The group is distributed from the southern United States to Argentina, and its species inhabit both open and forested areas (Duellman and Schulte, 1992; Frost, 2020). The following characters have been proposed as diagnostic of this species group: extremely ossified and exostosed skulls, elevated (keratinized or not) cranial crests, dorsal skin with small and large tubercles, and lateral row of enlarged tubercles absent (Duellman and Schulte, 1992; Pramuk, 2006; Maciel et al., 2010). Maciel et al. (2010) and Vallinoto et al. (2010) studied the phylogenetic relationships in this species group. Maciel et al. (2010) included phenotypic (morphological and parotoid-macrogland secretions) and molecular (sequences of three mitochondrial and one nuclear genes) characters and found this group as monophyletic, being the sister taxon of the *Rhinella crucifer* Group. Alternatively, Vallinoto et al. (2010) found the *R. crucifer* Group nested within the *R. marina* Group. Sequeira et al. (2011) reported the occurrence of extensive unidirectional introgression between *R. diptycha* (as *R. schneideri*) and some populations of *R. marina* that could contribute to biased inferences in the phylogenetic relationships. More recently, Vallinoto et al. (2017) reevaluated this hypothesis by including additional samples and molecular markers and found a more complex scenario with no evident pattern of unidirectional introgression and a doubtful taxonomic status of some *R. marina* populations. Finally, based on a phylogenetic analysis using mitochondrial genes and morphometric data, Acevedo et al. (2016) resurrected *R. horribilis* for the western Andean populations previously considered *R. marina.* Recently Bessa-Silva et al. (2020) found evidence of interspecific nuclear differentiation between these species and a marked discordance between mitochondrial and nuclear phylogenetic inferences in the *R. marina* Group.

We included samples of several populations from all the currently recognized species of this group: *Rhinella achavali, R. arenarum, R. cerradensis, R. diptycha, R. horribilis, R. icterica, R. jimi, R. marina, R. poeppigii, R. rubescens*, and *R. veredas.* For *R. arenarum*, we also included samples of the populations historically assigned to the subspecies *R. arenarum mendocina* (see Laurent, 1969).

THE *RHINELLA SPINULOSA* GROUP

Nine species are currently assigned to this group, which are distributed in the Andean region from southern Ecuador to southern Argentina and Chile, except for *Rhinella achalensis,* which is endemic to the Sierras Pampeanas Centrales in central Argentina (Cei, 1972b; Pramuk and Kadivar, 2003). The species of this group are medium sized and have a moderately to lightly ossified skull that lacks dermal sculpturing and exostosis. They also have a marked sexual dimorphism in skin texture and coloration (Vellard, 1959; Cei, 1972a, 1972b; Duellman and Schulte, 1992). This group was recovered as monophyletic in the combined phylogenetic analysis of Pramuk (2006: fig. 4) but paraphyletic in the separate molecular or morphological analyses (Pramuk, 2006: figs. 1–3). Some subspecies have been recognized for the nominal species of this group, which is a putative species complex (Vellard, 1959; Cei, 1972a; Ferraro et al., 2018).

We included all recognized species of this group: *Rhinella achalensis, R. amabilis, R. arequipensis, R. arunco, R. atacamensis, R. limensis, R. rubropunctata, R. spinulosa* (including populations historically assigned to the subspecies *R. s. papillosa, R. s. spinulosa,* and *R. s. trifolium*), and *R. vellardi*. We were unable to sample populations assigned to two subspecies of *R. spinulosa*: *R. s. altiperuviana* and *R. s. flavolineata*.

THE "*RHINELLA VERAGUENSIS* GROUP"

This nonmonophyletic group is composed of 17 small- to medium-sized species, all of which occur in the cloud forest of the Andes from northern Peru to northern Argentina, excepting *Rhinella chrysophora*, a species from north-central Honduras (Cei, 1972a; Duellman and Schulte, 1992; Chaparro et al., 2007; Cusi et al., 2017; McCranie, 2017). Members of this group are morphologically diverse with terrestrial, semiaquatic, or arboreal habits.

The following characters have been considered diagnostic for the *Rhinella veraguensis* Group: skull with weak exostosis, cranial crests absent or weak, dorsal skin bearing small elevated tubercles, and a lateral row of enlarged tubercles in some species (Gallardo, 1961; Cei, 1972a; Duellman and Schulte, 1992; Pramuk, 2006). This group has been consistently recovered as nonmonophyletic (Pramuk, 2006; Chaparro et al., 2007; van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Moravec et al., 2014; Pyron, 2014; Cusi et al., 2017; Jetz and Pyron, 2018) and its definition and composition are problematic (see Pereyra et al., 2015; and comments regarding the definition of the *R. acrolopha* and *R. festae* Groups above). For purposes of description of the ingroup, *R. lilyrodriguezae* is included in this group (according to the phylogenetic relationships recovered by Cusi et al., 2017), although this species was assigned to the *R. festae* Group in the original description. We included samples of most species of this group (*R. amboroensis, R. arborescandens, R. chavin, R. fissipes, R. inca, R. justinianoi, R. leptoscelis, R. lilyrodriguezae, R. manu, R. multiverrucosa, R. nesiotes, R. quechua, R. rumbolli, R. tacana, R. veraguensis,* and *R. yanachaga*). The only unsampled species was *R. chrysophora*, which occurs in the Wet Forest in the central and western portions of the Cordillera Nombre de Dios, central-north Honduras. This species has not been observed since 1996 and is thought to be extinct (McCranie, 2017).

Species Unassigned to Any Group

Six species of *Rhinella* are not currently assigned to any group (for a history of previous group assignments see appendix 5). Among them, we included *R. ceratophrys, R. gallardoi*, and *R. sternosignata* in our phylogenetic analyses. The following species were not included in the phylogenetic analyses: (1) *R. cristinae* (Vélez-Rodríguez and Ruiz-Carranza, 2002), a species known only from the type locality (Vereda Tarquí, km 53–54 on road Altamira-Florencia, Municipio de Florencia, Departamento del Caquetá, Colombia) and collected in 1990 for the last time; (2) *R. gnustae* (Gallardo, 1967), which is only known from the holotype collected in 1925 from an imprecise locality (Rio Grande, Jujuy, Argentina; see Lavilla et al., 2002); and (3) *R. iserni* (Jiménez de la Espada, 1875), which is also known with certainty only from the holotype and its type locality is imprecise (Andes de Chanchamayo, Peru).

Molecular Data

Tissue Sampling

The molecular data were the main source of evidence in terms of both number of scored characters and sampled terminals. As one of the main goals of this paper was to test the monophyly of all the species groups of *Rhinella*, we attempted to obtain tissue samples from as many species as possible, with particular emphasis on putative nonmonophyletic species groups. Additionally, we included specimens from multiple populations of species that include recognized subspecies (e.g., *R. arenarum* and *R. spinulosa*), species that might represent species complexes (e.g., *R. dapsilis, R. margaritifera*, and *R. proboscidea*), and widely distributed taxa (e.g., *R. diptycha, R. marina*, and *R. veraguensis*) to evaluate their taxonomy. We included GenBank sequences only in cases where precise voucher number and locality data are provided, for specimens sequenced for at least the 16S rRNA gene. Besides, we made an effort to corroborate the identity of most relevant vouchers. A detailed list of all the terminals included in our analyses is given in appendices 1 and 2.

Laboratory Protocols

We extracted total genomic DNA from ethanol-preserved tissues (liver, muscle, or fingertips) using the Qiagen DNeasy kit. We carried out PCR amplifications in a total volume of 25 µl reactions using 0.2 µl Taq (Fermentas). The PCR protocol consisted of an initial denaturation step of 3 min. at 94° C followed by 35 (for mitochondrial genes) or 45 (for nuclear genes) cycles consisting of 30 seconds at 94° C for denaturation, 40 seconds at 48°–62° C for annealing, and 30–60 seconds at 72° C for extension, and a final extension step of 10–15 minutes at 72° C. We cleaned PCR-amplified products using 10U of Exonuclease plus 1U of alkaline phosphatase per reaction. We sequenced the products with an automatic sequencer ABI 3730XL (Applied Biosystems) in both directions to check for potential errors and nuclear polymorphisms. We processed the chromatograms using the software Sequencher version 4.5 (Gene Codes, Ann Arbor, MI) and edited the complete sequences with BioEdit (Hall, 1999). Sequences are deposited in GenBank under the accession numbers MW002838–MW003700.

Genotypic character sampling

The mitochondrially encoded loci sampled for the phylogenetic analyses include: (1) the 12S rRNA, the tRNA Valine, and the 16S rRNA genes (*12S-tRNA*^{Val}*-16S*; 2469 bp), (2) a fragment comprising the upstream section of the 16S rRNA gene and the tRNA Leucine, NADH dehydrogenase subunit 1, and tRNA Isoleucine genes (*16S-tRNA*^{Leu}*-ND1- tRNA*^{Ile}; 1305 bp), and (3) a fragment of cytochrome *b* gene (*cytb*; 700 bp), for a total of up to 4474 bp. The nuclear loci include: (1) the C-X-C motif chemokine receptor 4 gene (*cxcr4*; 676 bp), (2) the solute carrier family 8 member A1 gene (*slc8a1*; 715 bp), (3) the proopiomelanocortin gene (*pomc*; 559 bp), (4) two nonoverlapping fragments of the recombination activating 1 gene (*rag1*-a and *rag1*-b; 936 and 429 bp respectively), and (5) the rhodopsin gene (*rho*; 316 bp), for a total of 3631 bp. Primers and their sources are detailed in table 1.

For the parsimony total evidence and maximum-likelihood (ML) analyses (see below), the amount of sequence data analyzed per terminal ranged from 447 bp (*Rhinella gildae* URCA 12651 obtained from GenBank) to 8089 bp (*R. henseli* CFBH 20117), with a mean of 4378 bp per terminal. All the phylogenetic datasets employed in the analyses are available at https://doi.org/10.5531/sd.sp.46).

Phenotypic data

The phenotypic dataset consisted of direct observations on specimens and bibliographic information for 90 characters, scored for 106 terminals (84 from the ingroup, 22 from outgroups). The scoring was recorded using Mesquite version 3.51 (Maddison and Maddison, 2018). The dataset was assembled from the following character systems: 33 from adult osteology, 15 from hand and foot musculature, 3 from the tympanic middle ear, 1 from adult visceral anatomy, 15 from adult external morphology, 9 from larval external morphology, 3 from larval chondrocranium, 4 from embryonic external morphology, 6 from natural history, and 1 from cytogenetics. Phenotypic characters are described below (see List and Description of Characters); the phenotypic matrix is included as supplementary data 1 (available at https://doi.org/10.5531/sd.sp.46).

Cranial and postcranial osteology follows the terminology employed by Trueb (1973, 1993), that of cranial crests follows Mendelson (1997a), and hand and foot myology follows Blotto et al. (2020). Terminology for larval external morphology follows Altig and McDiarmid (1999) and the characterization of embryonic structures follows Nokhbatolfoghahai and Downie (2005, 2008). Osteology was studied in (1) cleared and double-stained specimens prepared following the techniques of Wassersug (1976), (2) dry skeletons, and (3) µ-CT scans (available for download at www.morphosource.org, Duke University). Additional information was obtained from detailed osteological descriptions in the literature (see appendix 3). Visualization and data processing of µ-CT images was done in MeshLab (Cignoni et al., 2008). For the study of myology, dissections of the hand and foot musculature were performed to remove superficial layers and observe successively deeper muscles as outlined by Blotto et al. (2020). Topical applications of the iodine/potassium iodide solution of Bock and Shear (1972) were used when necessary to enhance contrast. The remaining characters were scored from the literature, unless specified (see appendix 3).

We scored multiple states for uncertainty or ambiguity in the condition of a terminal (among some states, but not all the character states) for some characters (see Pol and Apesteguía, 2005). This way of scoring let us incorporate relevant information (mainly from descriptions obtained from the bibliography) even when descriptions were not detailed enough. For 19 series of transformation, we used composite coding (sensu Maddison, 1993), which minimizes the occurrence of inapplicable or missing entries (Pimentel and Riggins, 1987; Maddison, 1993; Strong and Lipscomb, 1999).

Phylogenetic Analyses

The final taxon sample for the phylogenetic analyses was defined by means of a series of preliminary analyses that clarified the situation of many problematic terminals. As hybridization and genetic introgression, both nuclear and mitochondrial, seem to be common in some species of *Rhinella* (Sequeira et al., 2011; Pereyra et al., 2016a; Vallinoto et al., 2017), we first performed exploratory analyses of mitochondrial (MD) and nuclear (ND) datasets independently to detect nuclear-mitochondrial discordance as indicative of putative genetic introgression. Subsequently, we performed a total evidence (TE) analysis (Kluge, 1989, 2004; Nixon and Carpenter, 1996) combining nonintrogressed nuclear and mitochondrial sequences and the phenotypic dataset (see details in appendix 2).

The phylogenetic analyses of each separate molecular dataset (nuclear and mitochondrial, see below) and the total evidence analysis were performed in TNT version 1.5 (Goloboff et al., 2008; Goloboff and Catalano, 2016). Gaps were considered as a fifth state in all parsimony analyses (nuclear, mitochondrial, and total evidence analyses) and all classes of transformation events were equally weighted. In addition, we performed a total evidence analysis considering gaps as missing data for comparisons with the maximum likelihood analysis (see below). Unless otherwise stated, all results shown refer to parsimony analyses in which gaps were treated as a fifth state. We favoured parsimony as optimality criterion because the cladogram that minimizes transformations to explain the observed variation is the simplest one, maximizes evidential congruence, and has the greatest explanatory power (Farris, 1983; Goloboff, 2003; Goloboff and Pol, 2005; Kluge and Grant, 2006; Wheeler et al., 2006). Sequences were aligned using the online software MAFFT v7 (Katoh and Toh, 2008; Katoh et al., 2019) under the strategy E-INS-i (for the *12S-tRNA*^{Val}*-16S* fragment) and L-INS-i or G-INS-i (for remaining fragments), with default parameters for gap opening and extension. These alignments were used for both phylogenetic analyses and clade supports estimations (see details below).

Separate Phylogenetic Analyses of Nuclear and Mitochondrial Sequences

Both nuclear and mitochondrial datasets were analyzed in TNT using "New Technology" searches and performing a combination of sectorial searches, ratchet, and tree fusing (Goloboff, 1999; Nixon, 1999), using the default settings for these strategies. Tree searches were performed until the consensus was stabilized 10 times, with a factor of 75 (see Goloboff, 1999; Giribet, 2005).

The strict consensus tree resulting from the analysis of all sampled taxa of the nuclear dataset (= ND) was poorly resolved (data not shown). A poor resolution of the consensus can be due to the effect of just a small number of wildcard or rogue taxa, which are those that assume varying phylogenetic positions in the most parsimonious trees (MPT) (Nixon and Wheeler, 1992; Wilkinson, 1996; Aberer et al., 2013; Goloboff and Szumik, 2015). To avoid including terminals that act as wildcard taxa due to the lack of evidence, we included only terminals with more than three nuclear sequenced fragments (see appendix 2). Although there is an imperfect relationship between missing data and wildcard behavior, we identified three loci as the critical number to obtain an informative and comparable consensus in preliminary analyses. After excluding terminals with fewer than three nuclear fragments from the dataset, we reanalyzed this restricted nuclear dataset (rND) to estimate the consensus tree and clade supports (see below). The mitochondrial dataset was analyzed using the same terminals as the restricted nuclear dataset (i.e., restricted mitochondrial dataset, rMD) and similar parameters of analysis (see above), to allow the comparison.

Total Evidence Analysis

For the TE analysis, we followed the strategy described above for the separate nuclear and mitochondrial analyses. In this analysis, we included: (1) all the nuclear sequences from the complete nuclear dataset, (2) all the mitochondrial sequences

TABLE 1

Primers used to amplify and sequence DNA in this study

See appendix 2 for gene abbreviations.

Genome	Gene	Primer	Direction	Primer sequence 5’→3’	Source
Mitochondrial	*12S-16S*	MVZ59	Forward	ATAGCACTGAAAAYGCTDAGATG	Graybeal, 1997
		Phe2-L	Forward	AAAGCATAACACTGAAGATGTTAAGATG	Wiley et al., 1998
		12S F-H	Reverse	CTTGGCTCGTAGTTCCCTGGCG	Goebel et al., 1999
		12S A-L	Forward	AAACTGGGATTAGATACCCCACTAT	Goebel et al., 1999
		tRNAval-H	Reverse	GGTGTAAGCGARAGGCTTTKGTTAAG	Goebel et al., 1999
		12Sm	Forward	GGCAAGTCGTAACATGGTAAG	Pauly et al., 2004
		L13	Forward	TTAGAAGAGGCAAGTCGTAACATGGTA	Feller and Hedges, 1998
		Titus I	Reverse	GGTGGCTGCTTTTAGGCC	Titus and Larson, 1996
		L2A	Forward	CCAAACGAGCCTAGTGATAGCTGGTT	Hedges, 1994
		H10	Reverse	TGATTACGCTACCTTTGCACGGT	Hedges, 1994
		AR	Forward	CGCCTGTTTATCAAAAACAT	Palumbi et al., 1991
		Wilkinson2	Reverse	GACCTGGATTACTCCGGTCTGA	Wilkinson et al., 1996
	nd1	16S-frog	Forward	TTACCCTRGGGATAACAGCGCAA	Wiens et al., 2005
		tMet-frog	Reverse	TTGGGGTATGGGCCCAAAAGCT	Wiens et al., 2005
		ND1 F1	Forward	AGCCATAATCATCTGAACC	Smith et al., 2005
		ND1 R1	Reverse	TCCTCCCTATCAAGGAGGTCC	Smith et al., 2005
	cytb	CytbDen3-L	Forward	AAYATYTCCRYATGATGRAAYTTYGG	Santos and Cannatella, 2011
		CytbDen1-H	Reverse	GCRAANAGRAAGTATCATTCNGGYTTRAT	Santos and Cannatella, 2011
Nuclear	*cxcr4*	CXCR4-C	Forward	GTCATGGGCTAYCARAAGAA	Biju and Bossuyt, 2003
		CXCR4-G	Reverse	AGGCAACAGTGGAARAANGC	Biju and Bossuyt, 2003
	pomc	POMC-1	Forward	GAATGTATYAAAGMMTGCAAGATGGWCCT	Wiens et al., 2005
		POMC-2	Reverse	TAYTGRCCCTTYTTGTGGGCRTT	Wiens et al., 2005
		POMC-2B	Reverse	GCATTYTTGAAAAGAGTCATTARTGGAGTCTG	Pramuk, 2006
	rag1a	MartFl1	Forward	AGCTGCAGYCARTAYCAYAARATGTA	Hoegg et al., 2004
		AmpR1	Reverse	AACTCAGCTGCATTKCCAATRTCA	Hoegg et al., 2004
	rag1b	R1-GFF	Forward	GAGAAGTCTACAAAAAVGGCAAAG	Faivovich et al., 2005
		R1-GFR	Reverse	GAAGCGCCTGAACAGTTTATTAC	Faivovich et al., 2005
		RAG1 TG1F	Forward	CCAGCTGGAAATAGGAGAAGTCTA	Grant et al., 2006
		RAG1 TG1R	Reverse	CTGAACAGTTTATTACCGGACTCG	Grant et al., 2006
	rho	Rhod1A	Forward	ACCATGAACGGAACAGAAGGYCC	Bossuyt and Milinkovitch, 2000
		Rhod1C	Reverse	CCAAGGGTAGCGAAGAARCCTTC	Bossuyt and Milinkovitch, 2000
	slc8a1	NACAL	Forward	TCCAAAGCAGATATTGAAATGGA	Roelants and Bossuyt, 2005
		NACAO	Reverse	ATACCTGCATGATCATCATCAAA	Roelants and Bossuyt, 2005

from the complete mitochondrial dataset, and (3) the phenotypic dataset. The following criteria were used to treat putatively conspecific sequences as pertaining to the same or different terminals: (1) sequences from the same individual or conspecific individuals placed in well-supported discordant positions in the separate nuclear and mitochondrial analyses were considered as independent terminals, because discordance suggests mitochondrial introgression between different species (see Pereyra et al., 2016a); and (2) terminals from the phenotypic dataset were combined with the more closely related conspecific terminal of the molecular dataset (mitochondrial + nuclear). When mitochondrial and nuclear sequences of a specimen were included separately, the phenotypic data were combined with the nuclear sequences. Appendix 2 provides a list of all the terminals included and excluded in the TE analysis.

Resampling Support Measures

Two types of resampling support measures were estimated for the datasets in TNT version 1.5 (Goloboff and Catalano, 2016): (1) parsimony jackknife absolute frequencies (JAF; Farris et al., 1996) and (2) parsimony jackknife frequency differences (JGC; Goloboff et al., 2003). For estimation of both measures, we performed 1,000 replicates using "New Technology" searches consisting of a combination of sectorial searches, ratchet, and tree fusing (Goloboff, 1999; Nixon, 1999), reaching minimum length two times (preliminary analyses showed that minimum lengths are hit with this search strategy). Goloboff et al. (2003) noted that the resampling support for a clade does not necessarily correlate with the absolute frequency itself (i.e., the number of times a group is recovered in the resampled matrices), because groups with positive support (≥ 50%) can have much lower frequencies than groups with no support at all (<50%). To solve this situation, these authors proposed to also consider the value GC (i.e., frequency difference), which indicates the frequency differences between a group and the most frequent contradictory group. Values of this score range between -100% (maximum contradiction) and 100% (maximum support).

Maximum-Likelihood Analysis

Maximum-likelihood analysis was performed with IQ-TREE v1.6.12 (Nguyen et al., 2015) considering the same dataset (DNA sequences + phenotypic characters) as the TE analysis under parsimony. ModelFinder (Kalyaanamoorthy et al., 2017), which is implemented in IQ-TREE, was used to select the optimal partition scheme and substitution models for molecular characters. ModelFinder implements a greedy strategy (Lanfear et al., 2012) that starts with the full partition model and subsequentially merges two genes until the model fit does not increase any further. The best partition scheme included two subsets (see table 2). For morphological data we use the two morphological ML models (see Lewis, 2001) implemented in IQ-TREE (i.e., MK and ORDERED, for unordered and ordered characters respectively) considering the ascertainment bias correction (ASC) method. We consider edge-linked-proportional partition model but separate substitution models and rate evolution between partitions (-spp option). The maximum-likelihood tree was conducted with 1000 ultrafast bootstrap replicates (Minh et al., 2013; Hoang et al., 2018) using the option -bnni that reduces the risk of overestimating branch supports due to severe model violations. The resulting tree was visualized and edited in FigTree 1.4.3 (Rambaut, 2016). Partitions and models selected are detailed in table 2.

Taxonomic Evaluation

We considered the following criteria in assessing the taxonomic status of each lineage: (1) the cladogram topology resulting from the phylogenetic analyses, (2) the uncorrected pairwise distances (UPDs) of a fragment of the 16S rRNA gene (delimited by the primers AR and WILK2; see Vences et al., 2005a, 2005b; Fouquet et al., 2007b) calculated in PAUP* (Swofford, 2002), and (3) the known phenotypic evidence for each

TABLE 2

Best partition scheme and best-fit models selected by ModelFinder for the molecular data. For phenotypic data, we used morphological models considering the ascertainment bias correction (ASC) method.

Subset	Data blocks	Model
1	Coding mitochondrial sequences 1st, 2nd; Coding nuclear sequences 1st, 2nd, 3rd; Non coding mitochondrial sequences	GTR+F+I+G4
2	Coding mitochondrial sequences 3rd	TN+F+I+G4
3	Unordered phenotypic characters	MK+ASC
4	Ordered phenotypic characters	ORDERED+ASC

taxon. The phenotypic criterion was mainly considered in cases where relationships were unresolved (i.e. occurrence of polytomies) or poorly supported (JGC <50%) within a clade. For estimation of UPDs, datasets containing only sequences of the 16S rRNA gene for each species group (as are redefined in the Results section) were aligned in MAFFT under the strategy G-INS-i.

LIST AND DESCRIPTION OF CHARACTERS

Characters modified from previous phylogenetic studies are indicated with an asterisk (*).

Adult Osteology

Most of the osteological characters used here are those of Pramuk (2006), so they are not described in detail except when relevant (e.g., when character states were modified or additional character states were considered). Described characters refer to adult individuals of both sexes unless specified.

Skull

0. Preorbital crest (on the maxillary process of nasal), occurrence: (0) absent or indistinguishable, (1) weak, (2) well developed. **Additive**. Cranial crests were considered osteological characters, although it could also be scored from whole-preserved specimens. The use of presence/absence of cranial crests has a long history in bufonid taxonomy, and they were used in a phylogenetic context by Pramuk (2006: chars. 63–69). However, unlike Pramuk (2006), we differentiate between weak and well-developed crests. State 1 (weak) refers to cranial crests that are faint or not evident externally in living or intact preserved specimens, but evident in osteological preparations. State 2 (well developed) refers to crests that are evident externally in both intact and osteologically prepared specimens. When osteological preparations were not available to precisely determine the absent or weak state of the crest (since both states are similar in complete specimens) we scored these uncertainties as multiple states (i.e., 0/1, see Phenotypic data scoring in Material and methods section).

Previous usage in phylogenetic studies: Inger (1972: char. 29*), Morrison (1994: char. 13*), Mendelson (1997a: char. 6*), Pramuk (2006: char. 65*), Mendelson et al. (2011: char. 6*).

1. Supraorbital crest (on frontoparietals), occurrence: (0) absent or indistinguishable, (1) weak, (2) well developed. **Additive**.

Previous usage in phylogenetic studies: Inger (1972: char. 29*), Morrison (1994: char. 14*), Mendelson (1997a: char. 7*), Pramuk (2006: char. 68*), Mendelson et al. (2011: char. 7*).

2. Pretympanic crest (on the zygomatic ramus of squamosal), occurrence: (0) absent or indistinguishable, (1) weak, (2) well developed. **Additive**.

Previous usage in phylogenetic studies: Morrison (1994: char. 16*), Mendelson (1997a: char. 11*), Pramuk (2006: char. 66*), Mendelson et al. (2011: char. 11*).

3. Supratympanic crest (on the otic ramus of squamosal), occurrence in females: (0) supratympanic crest inconspicuous or developed, but that does not extend beyond the level of the cranial roof dorsally, (1) supratympanic crest hypertrophied extending beyond the level of the cranial roof dorsally. This character was codified separately for males and females since a dimorphic condition was detected. Large supratympanic crest occurs mainly in adult females of many species of the *Rhinella margaritifera* Group (Hoogmoed, 1990; Duellman and Schulte, 1992). However, males of some of these species also have large supratympanic crest (Hoogmoed, 1990).

Previous usage in phylogenetic studies: Morrison (1994: char. 17*), Mendelson (1997a: char. 10*), Vélez-Rodríguez (2004b: char. 35*), Pramuk (2006: char. 69*), Mendelson et al. (2011: char. 10*).

4. Supratympanic crest (on the otic ramus of squamosal), occurrence in males: (0) supratympanic crest inconspicuous or developed, but that does not extend beyond the level of the cranial roof dorsally, (1) supratympanic crest hypertrophied extending beyond the level of the cranial roof dorsally.

Previous usage in phylogenetic studies: Morrison (1994: char. 17*), Mendelson (1997a: char. 10*), Vélez-Rodríguez (2004b: char. 35*), Pramuk (2006: char. 69*), Mendelson et al. (2011: char. 10*).

5. Parietal crest (on frontoparietal), occurrence: (0) absent or indistinguishable, (1) weak, (2) well developed. **Additive**.

Previous usage in phylogenetic studies: Inger (1972: char. 29*), Morrison (1994: char. 15*), Mendelson (1997a: char. 8*), Pramuk (2006: char. 64*), Mendelson et al. (2011: char. 8*).

6. Nasals, shape of anterior margins: (0) relatively blunt, (1) acuminate.

Previous usage in phylogenetic studies: Mendelson (1997a: char. 34*), Scott (2005: char. 64*), Pramuk (2006: char. 4), Nussbaum and Wu (2007: char. 52*), Mendelson et al. (2011: char. 25*).

7. Nasals, medial contact: (0) not in contact medially, (1) in contact medially.

Previous usage in phylogenetic studies: Lynch (1978: char. 2*), Clarke (1981: char. 1*), Ford (1990: char. 1), Morrison (1994: char. 1*), Báez and Basso (1996: char. 2), Mendelson et al. (2000: char. 32), Scott (2005: char. 63), Fabrezi (2006: char. 1), Pramuk (2006: char. 3), Nussbaum and Wu (2007: char. 51), Ponssa (2008: char. 52*).

8. Contact between nasal and frontoparietal: (0) anterior margin of frontoparietal does not articulate with posterior margin of nasal (fig. 2A), (1) articulate only laterally (fig. 2B), (2) articulate along most of its margin but not completely (fig. 2C), (3) articulate along the entire margin (fig. 2D). **Additive**.

Previous usage in phylogenetic studies: Heyer and Liem (1976: char. 2*), Morrison (1994: char. 6*), Mendelson (1997a: char. 2*), Pugener et al. (2003: char. 12* [adult morphological characters]), Pramuk (2006: char. 8*), Mendelson et al. (2011: char. 2*).

9. Dermal roofing bones, sculpturing: (0) dermal bones of the skull completely smooth, (1) lightly exostosed, (2) heavily ornamented with pits, striations, and rugosities. **Additive**. Hyperossification in anurans involves the level of sculpturing and the number and identity of exostosed bones (see revision by Blotto et al., 2021). Although species of *Rhinella* display a relatively high diversity of hyperossification, for the time being, we scored the variation only in the dermal roofing bones (nasals and frontoparietal), until more detailed analyses of the skull morphology are carried out.

Previous usage in phylogenetic studies: Clarke (1981: char. 2*), Ford (1990: char. 4*), Morrison (1994: char. 11), Mendelson et al. (2000: char. 28*), Pugener et al. (2003: char. 10* [adult morphological characters]), Scott (2005: char. 61*), Fabrezi (2006: char. 2*), Pramuk (2006: char. 2), Nussbaum and Wu (2007: char. 62*).

10. Occipital artery pathway, coverage with bone: (0) occipital canal not covered by bone, (1) partially covered, (2) completely covered with bone. **Additive**.

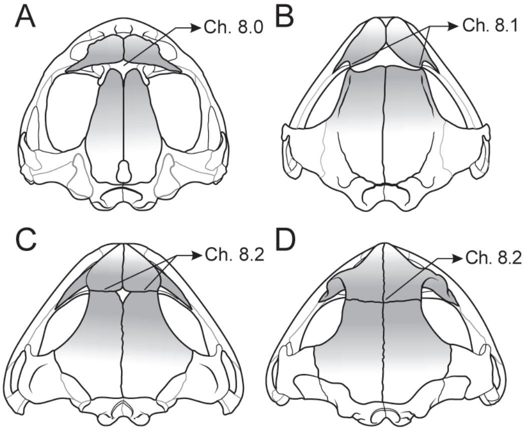

FIG. 2. Skulls (dorsal view) showing the different level of contact between nasals and frontoparietals (both bones in gray): **A,** *Nannophryne cophotis* KU 218525 (char. 8.0; species not included in this study); **B,** *Rhinella yanachaga* MUSM 24509 (char. 8.1); **C,** *R. crucifer* KU 93112 (char. 8.2); **D,** *R. marina* KU 152914 (char. 8.3). Panels A, C, D redrawn from Pramuk (2006), B redrawn from Lehr et al. (2007).

Previous usage in phylogenetic studies: McDiarmid (1971: char. 7*), Inger (1972: char. 10*), Heyer and Liem (1976: char. 3*), Lynch (1978: char. 4*), Clarke (1981: char. 4*), Morrison (1994: char. 10*), Mendelson (1997a: char. 4*), Mendelson et al. (2000: char. 38*), Pugener et al. (2003: char. 15* [adult morphological characters]), Wiens et al. (2005: char. 16*), Pramuk (2006: char. 9), Mendelson et al. (2011: char. 4*).

11. Squamosal, medial extension of otic ramus: (0) otic ramus of squamosal present, but not enlarged, (1) otic ramus of squamosal slightly enlarged, overlapping with the dorsal surface of the crista parotica, (2) otic ramus enlarged, in contact with posterolateral margin of frontoparietal, forming a continuous temporal arcade. **Additive**.

Previous usage in phylogenetic studies: Inger (1972: char. 12*), Lynch (1978: char. 5*), Clarke (1981: char. 5*), Ford (1990: char. 29*), Báez and Basso (1996: char. 16*), Mendelson (1997a: char. 33*), Faivovich (2002: char. 4*), Scott (2005: char. 65*), Wiens et al. (2005: char. 15*), Fabrezi (2006: char. 10*), Pramuk (2006: char. 15), Nussbaum and Wu (2007: char. 85*), Araujo-Vieira et al. (2019: char. 28*).

12. Nasals, extension of anterior margin: (0) anterior margins extend beyond the dorsal margins of the alary processes of the premaxillae (fig. 3A), (1) anterior margins are flush with the dorsal margins of the alary processes (fig. 3B), (2) anterior margins lie posterior to the dorsal margins of the alary processes (fig. 3C). **Additive**.

Previous usage in phylogenetic studies: Pramuk (2006: char. 21), Ponssa (2008: char. 57*).

13. Premaxilla, orientation of alary process: (0) angled posteriorly to the anterior margin of the premaxillae (fig. 4A), (1) dorsally projected to the anterior margin of the premaxillae (fig. 4B), (2) angled anteriorly to the anterior margin of the premaxillae (fig. 4C). **Additive**.

Previous usage in phylogenetic studies: Ford (1990: char. 12), Morrison (1994: char. 42), Mendelson (1997a: char. 23), Scott (2005: char. 78), Pramuk (2006: char. 26), Nussbaum and Wu (2007: char. 68), Ponssa (2008: char. 33), Barrionuevo (2017: char. 6*), Araujo-Vieira et al. (2019: char. 19*).

14. Septomaxilla, level of development of the anterior end: (0) not developed, (1) very developed and exposed anteriorly to the alary process of the premaxilla. Alcalde (2017) showed that bones previously described as "rostrals" (Pregill, 1981) or "prenasals" (Pramuk, 2000, 2006) in some bufonids are actually part of the

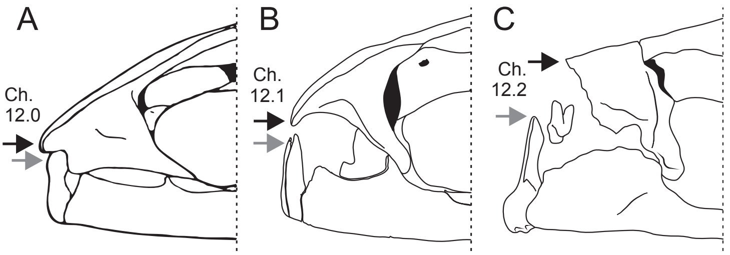

FIG. 3. Skulls (lateral view of the anterior region) showing the relation between the anterior margin of the nasal (black arrow) and the dorsal margin of the alary process of the premaxilla (gray arrow): **A,** *Rhinella yanachaga* MSM 24509 (char. 12.0), **B,** *R. amabilis* KU 124587 (char. 12.1), **C,** *Schismaderma carens* USNM 153380 (char. 12.2). Panels A and B redrawn from Lehr et al. (2007) and Pramuk (2006), respectively. Black arrows indicate the anterior margin of the nasal, gray arrows indicate the dorsal margin of the alary process.

enlarged and exposed anterior ends of the septomaxillae (and thus char. 42 of Pramuk [2006] refers to this structure instead to prenasals bones). Alcalde (2017) also pointed out the presence of an unpaired bone in the anterior end of the snout in *Rhinella dorbignyi* (as *R. fernandezae*, from the *R. granulosa* Group). He stated that it is homolog to the prenasal bone in some Lophyohylini (Hylidae; Trueb, 1970); even if primary homologs, they clearly represent independent instances of evolution. We observed this element in *R. beebei* (USNM 566017–8), but we could not determine its occurrence in other species of the group for which we do not consider this bone as a different character (see comments on the preservation and identification of this structure in Alcalde, 2017).

Previous usage in phylogenetic studies: Pramuk (2006: char. 42*).

15. Squamosal, articulation of zygomatic and ventral rami: (0) the zygomatic ramus of the squamosal is free from the ventral ramus, (1) the zygomatic ramus of the squamosal articulates with the ventral ramus of the squamosal.

Previous usage in phylogenetic studies: Mendelson (1997a: char. 32*), Vélez-Rodriguez (2004b: char. 32*), Pramuk (2006: char. 14*).

16. Jaw articulation: (0) posterior to the fenestra ovalis, (1) opposite to the fenestra ovalis, (2) anterior to the fenestra ovalis. **Additive**.

Previous usage in phylogenetic studies: Pramuk (2006: char. 25), Báez et al. (2012: char. 39).

17. Supraorbital flange on the frontoparietals: (0) frontoparietal does not extend laterally beyond the lateral margin of the sphenethmoid, (1) frontoparietal extends laterally beyond the lateral margin of the sphenethmoid.

Previous usage in phylogenetic studies: Morrison (1994: char. 4), Mendelson (1997a: char.1), Mendelson et al. (2000: char. 36), Pugener et al. (2003: char. 13 [adult morphological characters]), Wiens et al. (2005: char. 13), Pramuk (2006: char. 72), Mendelson et al. (2011: char. 1).

18. Sphenethmoid, extent of anterior ossification: (0) bony sphenethmoid reaches the level of palatines, but not beyond, (1) bony sphenethmoid beyond palatines, but does not reach the level of the premaxillae, (2) bony sphenethmoid reaches the level of the premaxillae anteriorly. **Additive**.

Previous usage in phylogenetic studies: Morrison (1994: char. 20), Mendelson (1997a: char. 13*), Vélez-Rodriguez (2004b: char. 21*), Pramuk (2006: char. 34*), Araujo-Vieira et al. (2019: char. 9*).

19. Pterygoid, articulation of the anterior ramus with maxilla: (0) anterior ramus of pterygoid articulates along the margin of maxilla, but does not contact with the palatine, (1) anterior ramus of pterygoid articulates along the margin of maxilla and contacts the palatine.

Previous usage in phylogenetic studies: Ford (1990: char. 32*), Morrison (1994: char. 52),

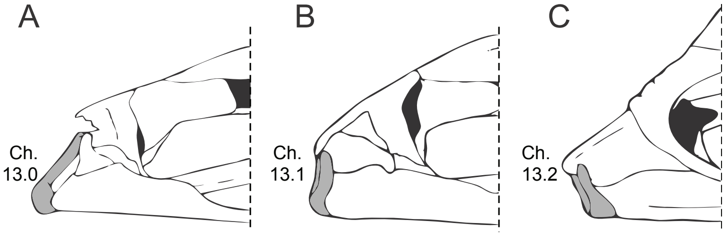

FIG. 4. Skulls (lateral view of the anterior region) showing the orientation of alary process of the premaxilla in relation to the anterior margin of the premaxilla (premaxilla in gray): **A,** *Nannophryne cophotis* KU 218525 (char. 13.0; species not included in this study; **B,** *R. crucifer* KU 93112 (char. 13.1); **C,** *R.* sp. *margaritifera* Group (char. 13.2). All the figures redrawn and slightly modified from Pramuk (2006). The voucher number provided for the specimen of the *R.* sp. *margaritifera* Group was erroneously stated in Pramuk's (2006) figures according to the information provided in appendix 1 of that publication and in VertNet database (http://portal.vertnet.org/).

Clarke (1981: char. 13*), Mendelson (1997a: char. 28*), Pugener et al. (2003: char. 40* [adult morphological characters]), Vélez-Rodríguez (2004b: char. 17*), Ponssa (2008: char. 67), Barrionuevo (2017: char. 29*).

20. Palatine, ventral ridge: (0) absent or indistinguishable, (1) present.

Previous usage in phylogenetic studies: Inger (1972: char. 18*), Morrison (1994: char. 33*), Mendelson (1997a: char. 15*), Mendelson et al. (2000: char. 10*), Pramuk (2006: char. 38), Mendelson et al. (2011: char. 14*).

21. Pterygoid, contact of medial ramus with ala of parasphenoid: (0) the medial ramus of the pterygoid is not in contact nor fused with the anterolateral margin of the ala of the parasphenoid, (1) the medial ramus of the pterygoid is fused with the anterolateral margin of the parasphenoid, (2) the medial ramus of the pterygoid is fused and extends medially along approximately half the length of the parasphenoid ala. **Additive**.

Previous usage in phylogenetic studies: Lynch (1978: char. 9*), Clarke (1981: char. 14*), Ford (1990: char. 34*), Morrison (1994: char. 54), Báez and Basso (1996: char. 28*), Mendelson (1997a: ch 29*), Vélez-Rodríguez (2004b: char. 19*), Pramuk (2006: char. 19).

22. Pterygoid, suture between the medial ramus and parasphenoid alae: (0) the surface of contact is smooth, (1) jagged or scalloped. This character is not applicable for specimens where the medial ramus of the pterygoid is not in contact or not fused with the anterolateral margin of the ala of the parasphenoid (char. 21.0).

Previous usage in phylogenetic studies: Grandison (1981: char. 13*), Pramuk (2006: char. 31).

23. Parasphenoid, shape of anterior margin of cultriform process: (0) acute and narrow (fig. 5A), (1) broadly rounded anteriorly (fig. 5B), (2) truncated (fig. 5C), (3) jagged or scalloped (fig. 5D). **Nonadditive**.

Previous usage in phylogenetic studies: Clarke (1981: char. 12*), Ford (1990: char. 45*), Morrison (1994: char. 36*), Mendelson (1997a: char. 20*), Scott (2005: char. 54*), Pramuk (2006: char. 29*), Nussbaum and Wu (2007: char. 98*), Araujo-Vieira et al. (2019: char. 42*).

24. Bony protrusion at the angle of jaws: (0) absent or indistinguishable, (1) weak, (2) developed into a processus. **Additive**. A bony protrusion ("or bony knob") is caused by a variable level of thickening of the ventrolateral margin of the quadratojugal. The level of development of the bony protrusion could also be determined both in living or intact specimens as in osteological preparations.

Previous usage in phylogenetic studies: Vélez-Rodriguez (2004b: char. 36*).

25. Hyoid, posterior lobe of the anterolateral process: (0) absent or indistinguishable (fig. 6A), (1) present (fig. 6B).

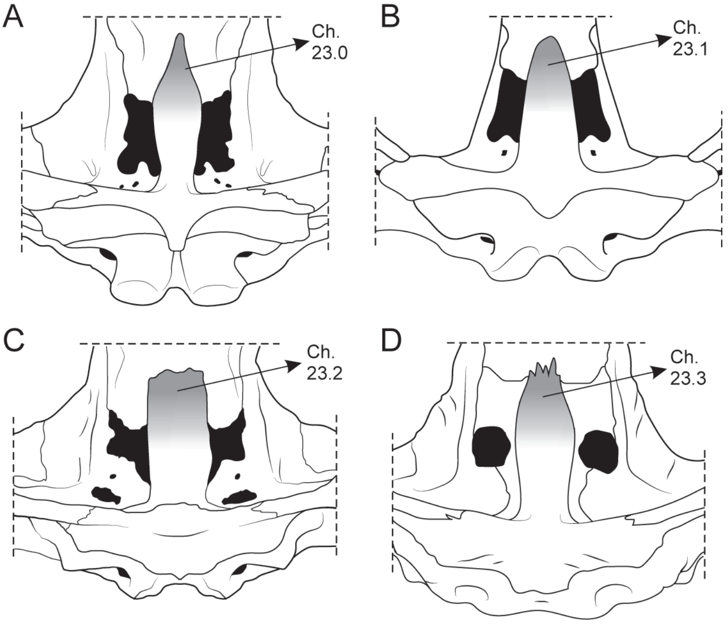

FIG. 5. Skulls (ventral view) showing the anterior margin of cultriform process of the parasphenoid (in gray): **A,** *Rhinella marina* KU 152914 (char. 23.0); **B,** *Nannophryne cophotis* KU 218525 (char. 23.1); **C,** *R. festae* USNM 167168 (char. 23.2); **D,** *R. cristinae* ICN 26233 (char. 23.3). Panels redrawn from Pramuk, 2006 (A, B); Trueb, 1971(C) and Vélez-R. and Ruiz-C., 2002 (D).

Previous usage in phylogenetic studies: Vélez-Rodriguez (2004: char. 42).

Vertebral Column

26. Presacral vertebrae, level of development of neural spine: (0) neural spine flat or slightly elevated, (1) neural spine notably elevated, protruding externally. The level of development of the neural spines can be determined both in intact-preserved specimens and in osteological preparations.

Previous usage in phylogenetic studies: Vélez-Rodriguez (2004b: char. 40*).

27. Presacral vertebrae, number: (0) eight, (1) seven. This number refers to the number of vertebrae even if there is some level of fusion between them. The number can be traceable even when there is fusion of centra due to the persistence of the intervertebral foramina (see Trueb, 1973; Cannatella, 1986).

Previous usage in phylogenetic studies: McDiarmid (1971: char. 23*), Lynch (1973: char. 1*), Grandison (1981: char. 15*), Cannatella (1986: char. 3*), Morrison (1994: char. 65*), Báez and Basso (1996: char. 30*), Wiens et al. (2005: char. 51*), Fabrezi (2006: char. 34), Pramuk (2006: char. 44*), Nussbaum and Wu (2007: char. 139), Mendelson et al. (2011: char. 43).

28. Presacral vertebrae I and II, fusion: (0) absent, (1) present. The fusion of the centra of both vertebrae into a single element may be identified for the occurrence of transverse processes and two foramina for vertebral nerves in the anterior presacral element.

Previous usage in phylogenetic studies: McDiarmid (1971: char. 24*), Lynch (1973: char. 2), Heyer and Liem (1976: char. 9), Cannatella (1986: char. 4*), Ford (1990: char. 66), Morrison (1994: char. 66), Wiens et al. (2005: char. 50), Grant et al. (2006: char. 145*), Nussbaum and

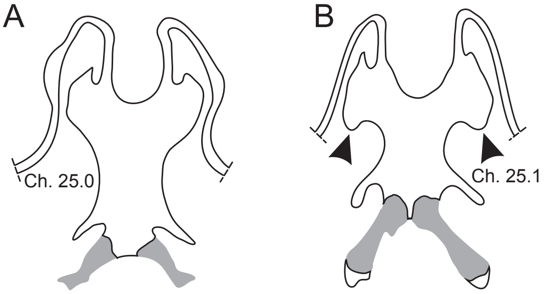

FIG. 6. Hyoid plate: **A,** *Rhinella cristinae* ICN 26233 (char. 25.0), **B,** *R. manu* MHNC 4404 (char. 25.1). Arrowheads indicate the occurrence of posterior lobes of the anterolateral processes in B. Panels redrawn from Vélez-R. and Ruiz-C., 2002 (A); Chaparro et al., 2007 (B).

Wu (2007: char. 137), Báez et al. (2012: char. 49*), Barrionuevo (2017: char. 43).

29. Sacrum, shape of sacral diapophyses: (0) the maximum width of the sacral diapophysis is smaller than its maximum length, (1) the maximum width of the sacral diapophysis is equal to, or greater than, its maximum length.

Previous usage in phylogenetic studies: Heyer (1975: char. 34*), Heyer and Liem (1976: char. 12*), Ford (1990: char. 75*), Morrison (1994: char. 70*), Báez and Basso (1996: char. 36*), Faivovich (2002: char. 21*), Pugener et al. (2003: char. 57* [adult morphological characters]), Fabrezi (2006: char. 42*), Grant et al. (2006: char. 143*), Pramuk (2006: char. 51), Araujo-Vieira et al. (2019: char. 95*).

30. Sacrum, orientation of anterior edge of sacral diapophyses: (0) posterior to the midline axis of the vertebral column, (1) perpendicular to the midline axis of the vertebral column, (2) anterior to the midline axis of the vertebral column. **Additive**.

Previous usage in phylogenetic studies: Scott (2005: char. 16), Pramuk (2006: char. 52), Nussbaum and Wu (2007: char. 142).

31. Sacrum and urostyle, fusion: (0) absent, (1) present.

Previous usage in phylogenetic studies: McDiarmid (1971: char. 25*), Lynch (1973: char. 3), Ford (1990: char. 76), Pugener et al. (2003: char. 58* [adult morphological characters]), Wiens et al. (2005: char. 60*), Nussbaum and Wu (2007: char. 138), Báez et al. (2012: char. 51*).

32. Ilium, dorsal protuberance, level of development: (0) large and slightly anteriorly or more dorsally directed, (1) small, low, and laterally projected. Gómez and Turazzini (2016) comment on the morphological variation and taxonomic distribution of this structure in anurans.

Previous usage in phylogenetic studies: Clarke (1981: char. 21*), Morrison (1994: char. 87*), Scott (2005: char. 12*), Pramuk (2006: char. 54), Báez et al. (2012: char. 65*).

Adult Musculature

Foot (ventral surface)

33. Discrete superficial cutaneous tendons, occurrence: (0) absent, (1) present. Burton (2004: 212, 220) described briefly this group of superficial tendons and Blotto et al. (2020) formalized this name. We scored if the superficial tendons are discrete or if they are absent or transformed into a sheet of connective tissue or fascia over the plantar side of the foot. Additional studies are needed to determine whether this group of tendons must be considered as a whole (as here) or individual superficial cutaneous tendons of each digit should be treated as independent characters. See further comments in Blotto et al. (2020).

34. M. interphalangeus proximalis digiti V, medial slip, occurrence: (0) absent, (1) present. See Dunlap (1960), Burton (2001, 2004), and Blotto et al. (2020) for descriptions of the mm.

interphalangei of the foot and comments on its taxonomic distribution in Anura.

35. M. interphalangeus proximalis digiti V, lateral slip, occurrence: (0) absent, (1) present.

36. M. abductor brevis plantaris hallucis, occurrence: (0) absent, (1) present. See Burton (2001, 2004) and Blotto et al. (2017) for characterization of this muscle and taxonomic distribution in nonbufonid taxa.

Previous usage in phylogenetic studies: Burton (2004: char. 30*), Faivovich et al. (2005: char. 7), Hoyos et al. (2014: char. 44), Blotto et al. (2017: char. 1).

37. M. flexor digiti II (FDM II), position of the origin with respect to the m. intermetatarsalis 1 (IMT 1): (0) FDM II ventral to the IMT 1, (1) FDM II dorsal to the IMT 1, (2) FDM II ventral and dorsal to the IMT 1. **Nonadditive**. See Dunlap (1960: 42) for an account under the name of m. flexor teres (for the FDM II) and transversus metatarsus (for the m. intermetatarsalis).

38. M. interosseus cruris, presence of an additional origin from the tibiale: (0) absent, (1) present. Most species have both an origin from the tibiale and from the fibulare (Gaupp, 1896; Dunlap, 1960; Burton, 2004). Among bufonid taxa, state 0 was reported for *Atelopus* (see Dunlap, 1960: 30), under the name of m. intertarsalis. The only species from our sampling that has state 0 is *Rhinella paraguas.*

Foot (dorsal surface)

39. M. extensor digitorum longus (EDL), insertion on metatarsophalangeal joint of digiti IV: (0) absent, (1) present. We scored the insertion of the EDL in each digit as an independent character, contra Burton (2004: char. 48), as discussed by Faivovich et al. (2005: 201). We found informative variation for the insertions on digits IV and V (next char.). The insertions on the metatarsophalangeal joint of the digits IV and V may be by an independent tendon or through a common tendon with the m. extensor brevis superficialis, m. extensor brevis medius, and/or the m. dorsometatarsalis proximalis, a source of variation not considered in the present study. See Dunlap (1960) and Burton (2004) for descriptions and variation of the insertion of this muscle, under the name m. extensor digitorum communis longus. The intraspecific variation reported by Inger (1972: 103) for the absence/presence of the insertion on each digit should be further tested; only *Nannophryne variegata* from our sampling was studied from more than one specimen to test this potential intraspecific variation.

40. M. extensor digitorum longus, insertion on metatarsophalangeal joint of digit V: (0) absent, (1) present.

Previous usage in phylogenetic studies: Inger (1972: char. 26*).

41. M. extensor brevis medius hallucis, occurrence: (0) absent, (1) present. See Dunlap (1960: 52–53) for description and variation across Anura.

Previous usage in phylogenetic studies: Hoyos et al. (2014: char. 37).

42. Lateral m. dorsometatarsalis proximalis digiti IV, discrete and independent tendon inserting on the proximal interphalangeal joint of digit IV: (0) absent, (1) present. Dunlap (1960: 57) considered the muscles dorsometatarsales proximales and the dorsometatarsales distales (both as mm. extensores breves profundi) as the same muscle (see discussion in Blotto et al., 2020). This fact partially precludes the understanding of the variation and taxonomic distribution described by Dunlap (1960). On the other hand, the extensive study of Hylidae by Burton (2004: char. H) suggests a great intraspecific variation when considering the number of tendons of insertion of the mm. dorsometatarsales proximales III–V (as extensores breves profundi). In our sampling, all species have a tendon of the lateral m. dorsometatarsalis proximalis digiti IV inserting on the distal interphalangeal joint of digit IV, while *Rhinella crucifer* and *R. henseli* have an additional independent tendon of insertion on the proximal interphalangeal joint. In the light of the variation found in Bufonidae, as well as in other clades of Anura (B.L.B., personal obs.), we decided to tentatively consider each tendon to each interphalangeal joint as independent transformation series.

Hand (ventral surface)

43. Medial m. lumbricalis brevis digiti V, slip from distal carpal 3-4-5: (0) absent, (1) present. The medial m. lumbricalis brevis digiti V may have two slips, one from the distal carpals and the other one from the flexor plate/adjacent tendo superficialis digiti V; both with a common or independent insertions (Burton, 1998: 59; this study). Nevertheless, Burton (1998: char. 18) discarded further discussion and comparison of the nature of this muscle given the extreme degree of variation found within his sampling ("Leptodactylidae" s.l.).

Previous usage in phylogenetic studies: Burton (1998: char.18).

Hand (dorsal surface)

44. M. extensor digitorum, insertion on metacarpophalangeal joint of digiti III: (0) absent, (1) present.

Some species lack the insertion on the metacarpophalangeal joint of the digit III. This insertion may be through a common tendon after inserting on the dorsal fascia of other muscles (usually mm. extensores breves superficiales) or by an independent tendon (Burton, 1998; this study).

Previous usage in phylogenetic studies: Burton (1998: char. 22*).

45. M. extensor digitorum, insertion on metacarpophalangeal joint of digiti V: (0) absent, (1) present. The slip of the m. extensor digitorum to the digit V may have two insertions, one on the metacarpophalangeal joint and a second insertion on the lateral side of the metacarpal V. The presence of both insertions varies independently across Anura (B.L.B., personal obs.), for which we scored their presence as independent transformation series. Within the current sampling of Bufonidae, the lateral insertion on metacarpal V is invariably present, and thus variation is restricted to the presence of the insertion on the metacarpophalangeal joint. This insertion may be through a common tendon after insertion on the dorsal fascia of other muscles or by an independent tendon (Burton, 1998; Araujo-Vieira et al., 2019; this study).

Previous usage in phylogenetic studies: Araujo-Vieira et al. (2019: char. 171).

46. M. extensor carpi ulnaris, occurrence of a head from the radioulna: (0) absent, (1) present. This head was not previously reported in the literature. It originates from the distal half or quarter of the radioulna, laterally to the origin of the m. abductor pollicis longus. The head converges with the head from the humerus, which attaches to the ulnare and distal carpal 3–4–5 (fig. 7).

47. M. extensor carpi ulnaris, nature of the origin of the head from the radioulna: (0) fleshy (fig. 7B), (1) via a flat tendon (fig. 7D). This character is not applicable for specimens that lack a supplementary head from the radioulna (char. 46.0).

Tympanic Middle Ear Complex

Pereyra et al. (2016b) reported the range of variation in structures of the tympanic middle ear (i.e., columella, annulus tympanicus, and tympanic membrane) in Bufonidae and demonstrated its unique evolutionary pattern within Anura.

48. Columella, occurrence: (0) absent, (1) present.

Previous usage in phylogenetic studies: Grandison (1981: char. 1*), Cannatella (1986: char. 6*), Ford (1990: char. 11), Morrison (1994: char. 27), Mendelson (1997a: char. 38*), Pugener et al. (2003: char. 47* [adult morphological characters]), Scott (2005: char. 81), Pramuk (2006: char. 17), Nussbaum and Wu (2007: char. 67*), Mendelson et al. (2011: char. 27).

49. Annulus tympanicus, occurrence: (0) absent, (1) present.

Previous usage in phylogenetic studies: Inger (1972: char. 28*), Drewes (1984: char. 23*), Cannatella (1986: char. 8*), Scott (2005: char. 80*), Wiens et al. (2005: char. 35*), Nussbaum and Wu (2007: char. 66).

50. Tympanic membrane: (0) absent, (1) present.

Previous usage in phylogenetic studies: Inger (1972: char. 28*), Heyer (1975: char. 2*), Drewes (1984: char. 23*), Cannatella (1986: char.

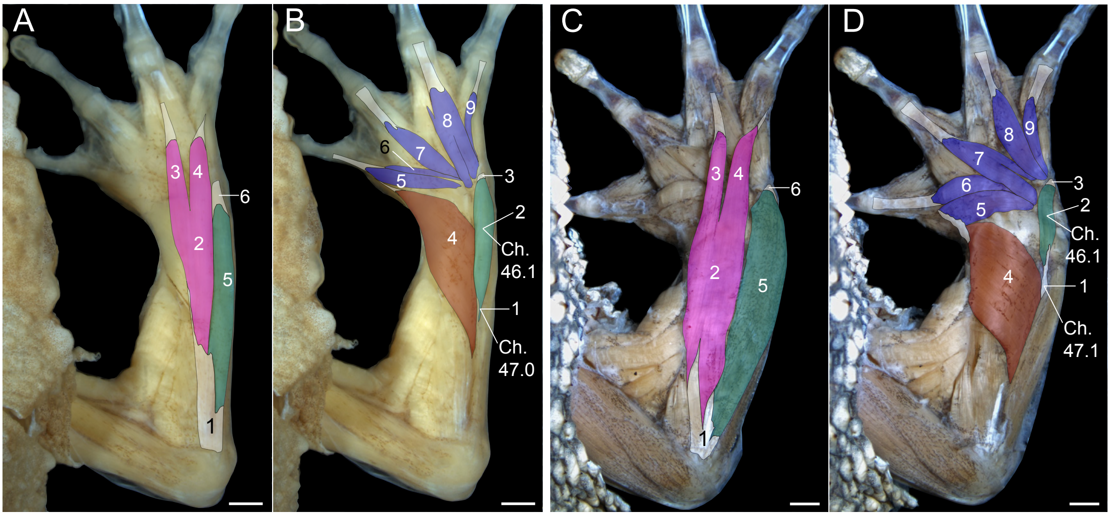

FIG. 7. Musculature of the dorsal surface of the hand and forearm: **A, B.** *Rhinella paraguas* CD 870. **C, D.** *R. dorbignyi* MACN 39350. **A.** First muscular layer: m. extensor digitorum; the m. extensor carpi ulnaris is also shown. Elements figured: **1,** m. extensor digitorum and the m. extensor carpi ulnaris (head from humerus): common tendon of origin; **2,** m. extensor digitorum; **3,** m. extensor digitorum: slip to the dorsal surface of the m. extensor brevis superficialis digiti IV (both muscles attaches to the metacarpophalangeal joint of digit IV via a common tendon); **4,** m. extensor digitorum: slip to metacarpal V; **5,** m. extensor carpi ulnaris: head from humerus; **6,** m. extensor carpi ulnaris (head from humerus): tendon of insertion. **B.** Second muscular layer: m. abductor pollicis longus and mm. extensores breves superficiales; the head from radioulna of the m. extensor carpi ulnaris is also shown (the head from humerus was removed). Elements figured: **1,** m. extensor carpi ulnaris (head from radioulna): fleshy origin; **2,** m. extensor carpi ulnaris: head from radioula; **3,** m. extensor carpi ulnaris (heads from humerus and radioulna): common tendon of insertion on distal carpal 3-4-5; **4,** m. abductor pollicis longus; **5,** m. extensor indicis brevis superficialis: slip from radiale; **6,** m. extensor indicis brevis superficialis: slip from ulnare; **7,** m. extensor brevis superficialis digiti III; **8,** m. extensor brevis superficialis digiti IV: slips from ulnare and distal carpal 3-4-5; **9,** m. extensor brevis superficialis digiti V. **C.** First muscular layer: m. extensor digitorum; the m. extensor carpi ulnaris is also shown. Elements figured: **1,** m. extensor digitorum and m. extensor carpi ulnaris (head from humerus): common tendon of origin; **2,** m. extensor digitorum; **3,** m. extensor digitorum: slip to the dorsal surface of the m. extensor brevis superficialis digiti IV (both muscles attaches to the metacarpophalangeal joint of digit IV via a common tendon); **4,** m. extensor digitorum: slip to metacarpal V; **5,** m. extensor carpi ulnaris: head from humerus; **6,** m. extensor carpi ulnaris: tendon of insertion. **D.** Second muscular layer: m. abductor pollicis longus and mm. extensores breves superficiales; the head from radioulna of the m. extensor carpi ulnaris is also shown (the head from humerus was removed). Elements figured: **1,** m. extensor carpi ulnaris (head from radioulna): tendon of origin; **2,** m. extensor carpi ulnaris: head from radioulna; **3,** m. extensor carpi ulnaris (heads from humerus and radioulna): common tendon of insertion on distal carpal 3-4-5; **4,** m. abductor pollicis longus; **5,** m. extensor indicis brevis superficialis: slip from ulnare inserting on metacarpal II (in common with the m. abductor pollicis longus); **6,** m. extensor indicis brevis superficialis: slip from ulnare inserting on metacarpophalangeal joint; **7,** m. extensor brevis superficialis digiti III: slips from ulnare and distal carpal 3-4-5; **8,** m. extensor brevis superficialis digiti IV: slip from distal carpal 3-4-5; **9,** m. extensor brevis superficialis digiti V. Characters figured: char. 46.1, presence of the head from radioulna of the m. extensor carpi ulnaris; char. 47.0, fleshy origin of the head from radioulna of the m. extensor carpi ulnaris; char. 47.1, origin via a flat tendon of the head from radioulna of the m. extensor carpi ulnaris. Scale bars = 1 mm.

8*), Morrison (1994: char. 96*), Scott (2005: char. 144*), Wiens et al. (2005: char. 108*), Ohler and Dubois (2006: char. 4*), Nussbaum and Wu (2007: char. 4), Barrionuevo (2017: char. 60).

Adult Visceral Anatomy

51. Inguinal fat bodies, occurrence: (0) absent, (1) present. Boulenger (1910) first reported the occurrence of elongated bodies associated to the muscles of the inguinal region in several species of Bufonidae. Later, Plytycz and Szarski (1987) and da Silva and Mendelson (1999) corroborated the occurrence of these inguinal fat bodies in many other species of several bufonid genera.

Previous usage in phylogenetic studies: Mendelson (1997a: char. 45), Pramuk (2006: char. 79), Mendelson et al. (2011: char. 34).

Adult External Morphology

All described characters of external morphology refer to adult individuals of both sexes, except when sexually dimorphic characters are considered (i.e., chars. 53–54 and 58–59).

52. Dorsal skin, macroscopic glandular structures in females: (0) indistinct; (1) small and smooth; (2) greatly enlarged and smooth; (3) with small tubercles, without cornified tip; (4) conical with a single cornified tip; (5) hemispherical with multiple cornified tips. **Nonadditive**. The skin of bufonids varies from completely smooth to highly tuberculated and warty due to modifications of dermal and/or epidermal components (Elias and Shapiro, 1959). It differs between sexes and its structure is affected (at least in males) by the reproductive condition of the specimens (see Cei, 1980; Duellman and Trueb, 1986). Therefore, when scoring skin diversity we considered: (1) as independent character structures present in both sexes (chars. 53, 54); (2) the maximum level of development reported for the dorsal structures of skin within the studied specimens; and (3) the most common structures present in the dorsal skin. Although some character states seem to be composed of a progressive series of transformation of glandular structures, detailed histological studies are required to understand the various components that are differentiated in each structure.

Previous usage in phylogenetic studies: McDiarmid (1971: char. 39*), Morrison (1994: char. 99*), Grant et al. (2006: char. 0*).

53. Dorsal skin, macroscopic glandular structures in males: (0) indistinct; (1) small and smooth; (2) greatly enlarged and smooth; (3) with small tubercles, without a cornified point; (4) conical with a single cornified point; (5) hemispherical with multiple cornified points. **Nonadditive**.

Previous usage in phylogenetic studies: McDiarmid (1971: char. 39*), Morrison (1994: char. 99*), Grant et al. (2006: char. 0*).

54. Vertebral line, occurrence: (0) absent, (1) present. Boulenger (1897) first noted the distinctiveness and independence between a filiform line or raphe and the vertebral line. The former is a line of thin skin extending along the middle of the back from the snout to the vent. This raphe is very common in bufonids and gives rise to a light vertebral line. As pointed out by Boulenger (1897), the independence of these structures is evident in cases of deviation of the former (see Boulenger, 1897: fig. 9). We consider the occurrence of a distinctive light vertebral line only.

Previous usage in phylogenetic studies: Heyer (1978: char. 1*), Ohler and Dubois (2006: char. 13*), Ponssa (2008: char. 1*).

55. Parotoid gland, occurrence: (0) absent, (1) present.

Previous usage in phylogenetic studies: Cannatella (1986: char. 11), Morrison (1994: char. 95*), Mendelson (1997a: char. 43*), Wiens et al. (2005: char. 109), Fabrezi (2006: char. 72), Pramuk (2006: char. 73*).

56. Parotoid gland, shape: (0) approximately ellipsoid, longer than wide (fig. 8A); (1) subtriangular (fig. 8B); (2) round to ovoid mostly symmetrical (fig. 8C); (3) triangular and bulky (fig. 8D). **Nonadditive**. This character is not applicable for specimens that lack a parotoid gland (char. 55.0).

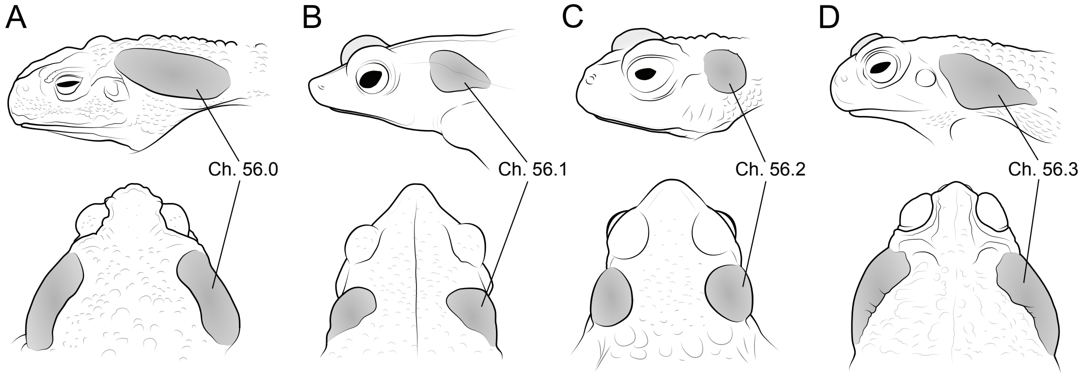

FIG. 8. Head (lateral and dorsal views) showing the shape of the parotoid gland (in gray): **A,** *Rhinella* aff. *cerradensis* (char. 56.0); **B,** *R. acutirostris* (char. 56.1); **C,** *R. arunco* (char. 56.2); **D,** *R. marina* (char. 56.3).

Previous usage in phylogenetic studies: Morrison (1994: char. 95*), Pramuk (2006: char. 73*), Mendelson et al. (2011: char. 32*).

57. Skin, occurrence of row of dorsolateral tubercles: (0) absent, (1) present.

Previous usage in phylogenetic studies: Morrison (1994: char. 97), Mendelson (1997a: char. 44*), Pramuk (2006: char. 81), Mendelson et al. (2011: char. 33).

58. Vocal sac, occurrence in adult males: (0) absent, (1) present. The vocal sac develops as ventral diverticula of the mouth floor into spaces among submandibular muscles (Noble, 1931; Tyler, 1971). This second cavity communicates with the buccal cavity via single or paired apertures, the vocal slits. In this way, the presence of a vocal sac automatically implies the presence of at least one vocal slit (and vice versa). Modifications of the gular skin (i.e. "internal" or "external" vocal sacs sensu Günther, 1858a) can be absent or present without affecting the codification of this character. Vocal sacs are either absent or present in adult males of the majority of species, with few exceptions where both states cooccur in different specimens (Liu, 1935; Inger and Greenberg, 1956, Hayes and Krempels, 1986; Mendelson, 1997b).

Previous usage in phylogenetic studies: Liem (1970: char. 36*), Drewes (1984: char. 20*), Cannatella (1986: char. 10*), Hillis and de Sá (1988: char. 6), Mendelson et al. (2000: char. 51*), Grant et al. (2006: char. 76*), Pramuk (2006: char. 75*), Ohler and Dubois (2006: char. 14), Mendelson et al. (2011: char. 31*).

59. Vocal slits, number: (0) unilateral, (1) bilateral. Several authors (e.g., Boulenger, 1897; Liu, 1935; Inger and Greenberg, 1956) reported the occurrence of specimens with a single vocal slit. This condition was observed in some species of Bufonidae and has not been reported in other anuran families. The single vocal slit can either be on the left or the right side of the tongue in different specimens of the same species. Furthermore, there are species where one (on either side) or two vocal slits can occur. This character is scored as not applicable for taxa lacking vocal sacs (see char. 58.0).

Previous usage in phylogenetic studies: Drewes (1984: char. 20*), Cannatella (1986: char. 10*), Mendelson (1997a: char. 42*), Mendelson et al. (2000: char. 51*), Pramuk (2006: char. 75*), Mendelson et al. (2011: char. 31*).

60. Vocal sac, shape when fully inflated: (0) spherical or subspherical, (1) projected anteriorly. Simple subgular vocal sacs are often spherical or subspherical. Nevertheless, in a few species, they project anteriorly deviating from a spherical shape. The degree of projection ranges from a slight deformation to a large, vertically oriented lobe. McAllister (1961) reported on this variation in North American bufonids and their putative relationship with vocalization, but this character has not been used in phylogenetic studies. This character is not applicable for specimens that lack a vocal sac (char. 58.0)

61. Nuptial pads, occurrence in males: (0) absent, (1) present. Nuptial pads are sexually dimorphic structures that can be present in the fingers of males; their structure and diversity were recently studied (Luna et al., 2018).

Previous usage in phylogenetic studies: Liem (1970: char. 35), Heyer (1975: char. 3*), Scott (2005: char. 132*), Wiens et al. (2005: char. 100), Grant et al. (2006: char. 23), Ohler and Dubois (2006: chars. 16–18*), Ponssa (2008: char. 24*), Barrionuevo (2017: char. 69).

62. Nuptial pads, coloration: (0) light colored, (1) dark colored. Following Luna et al. (2018) we distinguished between dark- and light-colored nuptial pads, where “dark-colored” includes all tones of brown and black and “light-colored” includes beige/uncolored pads. These differences in coloration result from minor changes in the stratum corneum of the epidermis and are independent of the number of layers of this stratum (Luna et al., 2008). This character is not applicable for specimens that lack nuptial pads (char. 61.0).

Previous usage in phylogenetic studies: Ohler and Dubois (2006: char. 24*).

63. Manus, occurrence of webbing between fingers: (0) absent or poorly developed, (1) present, well developed.

Previous usage in phylogenetic studies: Wiens et al. (2005: char. 99), Pramuk (2006: char. 77*), Nussbaum and Wu (2007: char. 12*).

64. Pes, edge of foot webbing: (0) smooth, (1) serrated.

Previous usage in phylogenetic studies: Vélez-Rodriguez (2004b: char. 12*).

65. Tarsus, occurrence of tarsal fold: (0) absent, (1) present. A tarsal fold is a dermal fold on the medial-ventral surface of the foot, extending proximally from the inner metatarsal tubercle.

Previous usage in phylogenetic studies: Inger (1972: char. 31*), Heyer (1975: char. 6*), Scott (2005: char. 156*), Grant et al. (2006: char. 28), Ohler and Dubois (2006: char. 11*), Ponssa (2008: char. 19), Barrionuevo (2017: char. 77).

66. Relative size of adult females and males: (0) adult females similar in size or larger than adult males, (1) adult males much larger than adult females. As a first approximation, we consider only two states due to the occurrence of a more evident gap in size according to published data. However, a more detailed study of sexual dimorphism in *Rhinella* could help to partition these into more additional states.

Previous usage in phylogenetic studies: Scott (2005: char. 139), Fabrezi (2006: char. 78*), Ponssa (2008: char. 110*).

Larval External Morphology

67. Body, morphology of the peribranchial and abdominal regions: (0) absence of external modifications, (1) presence of bulging regions lateral to the oral disc, (2) occurrence of an abdominal sucker. **Additive**. Most species of *Rhinella* have lentic larvae that lack external modifications in the peribranchial and abdominal regions (state 0). Modifications in these regions are typical of some lotic forms (McDiarmid and Altig, 1999; Hoff et al., 1999) and two different states occur within *Rhinella*. Larvae of *Rhinella rumbolli* have a central depression delimited by bulbous lateral regions in the peribranchial zone (state 1). Moreover, some other species of the *R. veraguensis* Group have a well-developed abdominal sucker that is bounded anteriorly by the oral disc, and the lateral and posterior edges are free from the body (state 2). We consider the character states to represent an ordered series of transformation for which the states are considered as additive.

68. Body, dorsal coloration: (0) light brown, (1) dark brown, (2) sharply defined dark markings on pale ground. **Nonadditive**.

69. Caudal musculature, ocurrence of an unpigmented longitudinal stripe along the inferior edge in the caudal musculature: (0) absent, (1) present. An unpigmented longitudinal stripe along the inferior edge of the caudal musculature sometimes occur in the caudal musculature of larvae having a dark coloration of the tail.

Previous usage in phylogenetic studies: Mendelson et al. (2011: char. 38*).

70. Caudal musculature, occurrence of irregular transverse whitish stripes: (0) absent, (1) present. In some species of the *Rhinella granulosa* and *R. veraguensis* Groups there are irregular transverse whitish stripes of variable extension due to the absence of melanocytes contrasting with the general dark coloration of the dorsal musculature (see Blotto et al., 2014, for taxonomic distribution in *Rhinella*).

71. Oral disc, occurrence of submarginal papillae: (0) absent, (1) present.

Previous usage in phylogenetic studies: Grant et al. (2006: char. 91*), Barrionuevo (2017: char. 86*), Araujo-Vieira et al., (2019: char. 135).

72. Oral disc, number of posterior labial tooth rows: (0) two, (1) three.

Previous usage in phylogenetic studies: Hillis and de Sá (1988: char. 2*), Wiens et al. (2005: char. 122*), Grant et al. (2006: char. 94*), Ohler and Dubois (2006: char. 31*), Barrionuevo (2017: char. 90*), Araujo-Vieira et al. (2019: char. 141*).

73. Oral disc, condition of the labial tooth row A2: (0) complete, (1) divided.

Previous usage in phylogenetic studies: Mendelson et al. (2011: char. 37*).

74. Oral disc, condition of the labial tooth row P1: (0) complete, (1) divided.

Previous usage in phylogenetic studies: Wiens et al. (2005: char. 124), Araujo-Vieira et al. (2019: char. 142)

75. Vent tube, opening: (0) medial, (1) dextral.

Previous usage in phylogenetic studies: Grant et al. (2006: char. 96*), Barrionuevo (2017: char. 93), Araujo-Vieira et al., 2019 (char. 145).

Larval Chondrocranium

Oliveira et al. (2014) studied the chondrocranium of some species of *Rhinella* and reviewed the information available for other bufonids.

76. Otic capsule, larval crista parotica, occurrence of processus anterolateralis: (0) absent or indistinguishable, (1) poorly developed with a rounded aspect, (2) well developed with an acute appearance. **Additive**.

Previous usage in phylogenetic studies: Larson and de Sá (1998: char. j*), Haas (2003: char. 66*); Miranda et al. (2015: char. 61*).

77. Procesus ascendens, angle of attaching to the braincase: (0) obliquely attached, (1) perpendicularly attached.

Previous usage in phylogenetic studies: Larson and de Sá (1998: char. o*), Miranda et al. (2015: char. 65*).

78. Copula anterior, occurrence: (0) absent, (1) present.

Previous usage in phylogenetic studies: Haas (2003: char. 105), Pugener et al. (2003: char. 35 [larval characters]), Miranda et al. (2015: char. 73).

Embryonic Morphology

Vera Candioti et al. (2016) studied the early ontogeny and described the informative variation found in several species of Bufonidae. All the characters considered on embryonic morphology were described in detail in that publication.

79. Third pair of external gills, condition: (0) absent or indistinguishable, (1) short, (2) long. **Additive**.

80. Dorsal line of hatching glands: (0) short (cephalic region only), (1) long (beyond cephalic region).

81. Type of adhesive gland: (0) A, (1) B.

82. Time of division of adhesive gland: (0) slightly after the second-gill pair branches off before operculum at the gill base, (1) immediately before the gills reach their maximum development, (2) immediately after opercular fusion. **Additive**.

Natural History

83. Diel activity of adults: (0) diurnal, (1) nocturnal.

Previous usage in phylogenetic studies: Grant et al. (2006: char. 115*).

84. Habits: (0) terrestrial, (1) arboreal, (2) aquatic. **Nonadditive.** Some species of the *Rhinella veraguensis* Group are completely arboreal. We do not consider as arboreal the mostly terrestrial species that have the ability to climb up the vegetation to rest during the night (de Noronha et al., 2013).

Previous usage in phylogenetic studies: Grant et al. (2006: char. 114*).

85. Oviposition site: (0) aquatic, (1) terrestrial, (2) phytotelmata. **Nonadditive**. Following van Bocxlaer et al. (2010), terrestrial oviposition refers to eggs that are placed on the ground, in leaf litter, or under stones, and are exposed to little or no free water at the time of oviposition. Phytotelmata refers to any chambers in a plant that is used as oviposition site (e.g., water-filled nut, tree holes, leaf axils; see Lehtinen et al., 2004; Grant et al., 2006).

Previous usage in phylogenetic studies: Faivovich (2002: char. 83*), Grant et al. (2006: char. 107*), Araujo-Vieira et al. (2019: char. 191*).

86. Structure of the spawn: (0) strings, (1) open clump, (2) mass, (3) strands. **Nonadditive**. Altig and McDiarmid (2007) reviewed in detail the terminology and diversity of arrangement of deposited eggs in Amphibia.

Previous usage in phylogenetic studies: Haas (2003: char. 141*).

87. Egg disposition in strings: (0) uniserial, (1) biserial, (2) multiserial. **Nonadditive**. Mature oocytes are surrounded by jelly layers as they are displaced through the different regions of the oviduct (Salthe, 1963; Altig and McDiarmid, 2007). The number and type of jelly layers are not well characterized in *Rhinella* (Pereyra et al., 2015), and there is no information about a direct relation between the diversity of strings and the eggs disposition within the string. Thus, we cannot infer a series of transformation and we consider this character as nonadditive.

88. Ovum pigmentation: (0) unpigmented, (1) animal pole pigmented.

Previous usage in phylogenetic studies: McDiarmid (1971: char. 42*), Grandison (1981: char. 4*), Cannatella (1986: char. 14*), Grant et al. (2006: char. 68*), Ohler and Dubois (2006: char. 29), Mendelson et al. (2011: char. 40*).

Cytogenetics

89. Nucleolar Organizer Regions, location: (0) terminal position of the short arms of the chromosome pair 1, (1) pericentromeric position of the long arms of the chromosome pair 1, (2) terminal position of the long arms of the chromosome pair 5, (3) terminal position of the long arms of the chromosome pair 6, (4) interstitial position of the short arms of the chromosome pair 7, (5) interstitial position of the long arms of the chromosome pair 10, (6) terminal position of the long arms of the chromosome pair 10, (7) interstitial position of the short arms of the chromosome pair 11. **Nonadditive**.

Previous usage in phylogenetic studies: Faivovich (2002: char. 82*).

RESULTS

Separate Analyses of Restricted Nuclear (rND) and Mitochondrial (rMD) Datasets

The parsimony analyses, reaching a stable consensus 10 times, retained 706 unique MPTs of length 1757 for the rND and one MPTs of length 11,436 for the rMD. Within the ingroup (i.e., *Rhinella*), the main incongruence between the rND and rMD analyses involved the position of the specimens of *R. horribilis*, which are deeply nested within the *R. marina* Group in the rND analysis, but were recovered as the sister clade of the *R. marina* + *R. crucifer* Groups in the rMD analysis (fig. 9). Based on these observations and previous published results (Pereyra et al., 2016a), we included the mitochondrial and nuclear genomes of *R. bernardoi* and *R. horribilis* as independent terminals in the TE analysis (see Discussion section for comments on the putative mitochondrial or nuclear introgression in these terminals and the rationale for the considerations of both genomes as independent terminals). Mitochondrial introgression and hybridization between *R. diptycha* and *R. marina* might have occured in the area south of the Amazon River (see Sequeira et al., 2011), but the evidence is not conclusive (see Vallinoto et al., 2017). For this reason, we did not include sequences of these species from this complex area. In appendix 2, we list the terminals considered in the TE analysis.

Total Evidence Analysis

Molecular data were included for all 320 terminals of 124 species, whereas phenotypic data were restricted to 106 specimens of 102 species (90 characters; ~50 scores/terminal). The TE analysis using parsimony, reaching a stable consensus 10 times, retained 657 unique MPTs (length 25,399). One of the optimal topologies is shown in figures 10–14 (fig. 10 for outgroup relationships, figs. 11–14 for *Rhinella* relationships). A summary tree of *Rhinella* relationships to species level is shown in the supplementary data 2 (available at https://doi.org/10.5531/sd.sp.46). In depicting all unrefuted clades, we employ the strict consensus of the optimal phylogenetic hypotheses resulting from this TE analysis treating gaps as fifth state as the basis of our discussion of taxonomy. The results of the TE analysis considering gaps as missing data (see supplementary data 3.1–3.5, available at https://doi.org/10.5531/sd.sp.46) and the ML analysis (see supplementary data 4.1–4.5, available at https://doi.org/10.5531/sd.sp.46) were highly congruent with the TE analysis considering gaps as fifth state. The few differences between these hypotheses are discussed when relevant.

The MPTs resulting from the TE analysis recovered *Rhinella* as nonmonophyletic due to the position of *R. ceratophrys* that is the sister taxon of *Rhaebo nasicus* with strong support (JGC and JAF = 100%; see fig. 10). Among outgroups the strongly supported *Anaxyrus* + *Incilius* (JGC = 96%, JAF = 97%) is the sister clade of all the other species of *Rhinella*. The monophyly of the clade composed of these three genera is poorly supported (JGC = 63%, JAF = 73%). The species of *Rhinella* (excluding *R. ceratophrys*) are monophyletic, well supported (JGC and JAF = 98%), and grouped in two major clades. One of these is moderately supported (JGC = 88%, JAF = 92%) and includes the species of the former *R. spinulosa* Group (including *R. gallardoi*; see Discussion) and those of the *R. granulosa, R. crucifer,* and *R. marina* Groups (figs. 11, 12). The other is strongly supported (JGC and JAF = 99%) and composed of all the species from the nonmonophyletic *R. veraguensis* and *R. margaritifera* Groups, the former *R. acrolopha* Group (see Discussion section), and *R. sternosignata* (figs. 13, 14).

Uncorrected P-Distances

The patterns of UPDs found within each species group vary largely (see below), so we did not consider a single value as a threshold to delimit species, but each particular situation was considered in the context of the genetic distances found within each species group. Interspecific distances among all the species addressed by the taxonomic revision are presented in the Discussion section of each species group. Throughout the text the UPDs are expressed as percentage.

DISCUSSION

Systematics and Taxonomy

Relationships among Outgroups and *Rhinella*

Our outgroup sample was designed exclusively to provide a rigorous test of the monophyly of *Rhinella* and does not constitute a critical test of previously hypothesized relationships among other clades of Bufonidae (e.g., Frost et al., 2006; Pramuk, 2006; Pramuk et al., 2008; van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Portik and Papenfuss, 2015; Jetz and Pyron, 2018). Indeed, most of the basal relationships of Bufonidae are unresolved or poorly supported in the TE analysis (fig. 10). Nevertheless, we found *Anaxyrus* + *Incilius* to be the sister clade of *Rhinella* with low support

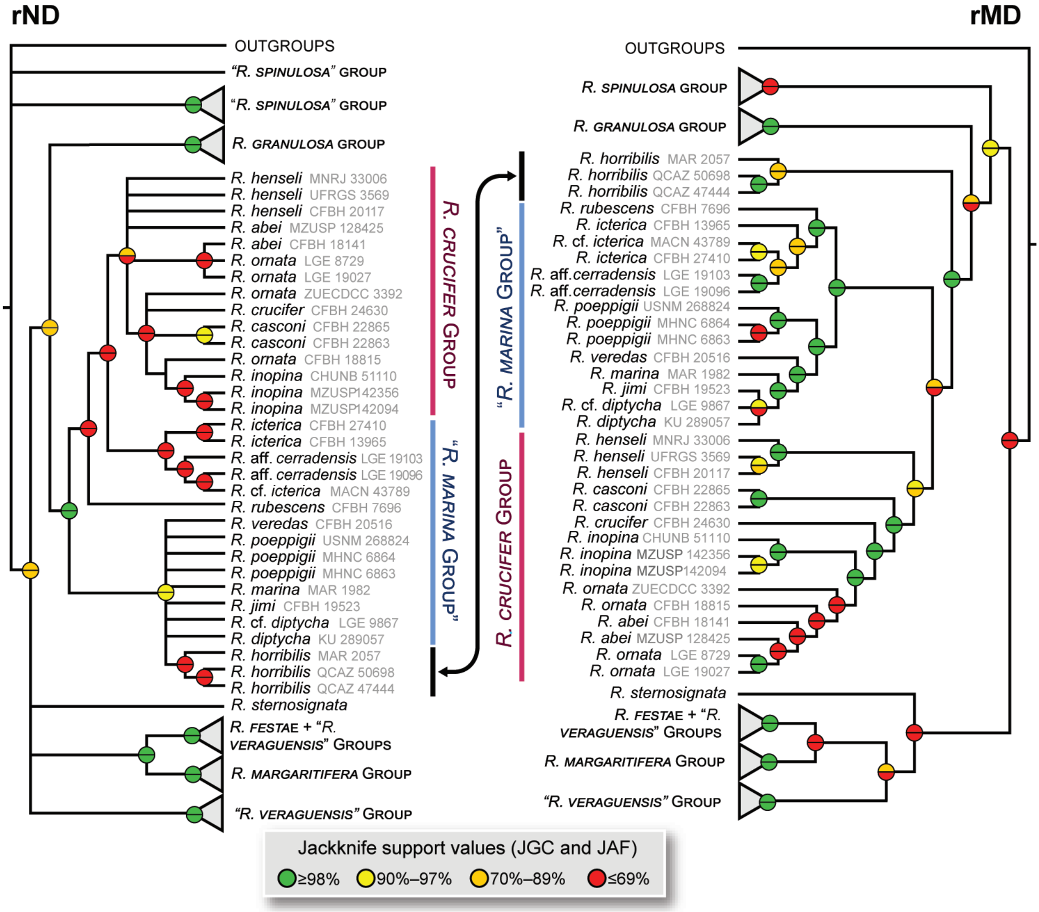

FIG. 9. Comparison between the strict consensuses resulting from the analyses of the restricted nuclear dataset (rND) and restricted mitochondrial dataset (rMD), showing the alternative positions of *Rhinella horribilis* in both analyses. Circles on nodes indicate parsimony jackknife frequencies (frequency differences value [above]/absolute [below]). Nodes lacking circles have <25% frequency difference values or < 50% jackknife absolute frequencies.

(JGC = 63%, JAF = 73%). The clade composed of these three genera, in turn, is the sister taxon of a large and poorly supported clade (JGC <25%, JAF <50%) of African and Eurasian bufonids. An almost identical relationship was recovered in the ML analysis (see supplementary data 4.1). The sister-group relationship between *Rhinella* and *Anaxyrus* + *Incilius* is consistent with the results of most previous phylogenetic analyses (e.g., Pramuk, 2006: fig. 4; Pramuk et al., 2008; Pyron and Wiens, 2011; Pyron, 2014: suppl. information "amph_shl.tre"; Portik and Papenfuss, 2015; Jetz and Pyron, 2018: suppl. information "amph_shl_new.tre"). Alternatively, van Bocxlaer et al. (2010: fig. S1) recovered *Rhinella* as the sister taxon of a clade comprising all African and Eurasian bufonids.

Although the vast majority of species of *Rhinella* form an exclusive clade, it is polyphyletic because *R. ceratophrys* was recovered as the sister taxon of *Rhaebo nasicus* with strong support (JGC and JAF = 100%). This relationship is not surprising, given that the morphological resemblance between both species was pointed out previously (e.g., Hoogmoed, 1977; Fenolio et al., 2012).

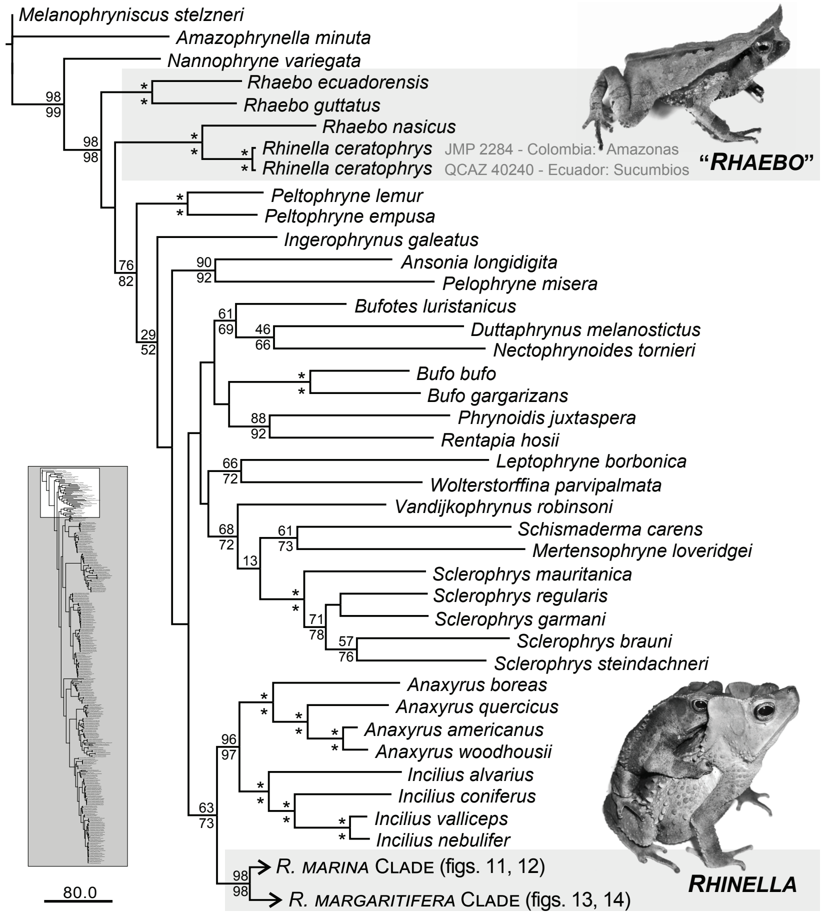

FIG. 10. Phylogenetic relationships of *Rhinella* and outgroups recovered in one of the most parsimonious trees from the total evidence analysis with TNT considering gaps as a fifth state (length 25,399 steps). Values around nodes are parsimony jackknife frequencies (frequency differences value [above]/absolute [below]). An asterisk (*) indicates 100% jackknife support. Clades lacking references have <25% frequency difference values or <50% jackknife absolute frequencies. Lower left inset shows the entire cladogram with present view marked in white.

Although *Rhaebo* was paraphyletic in our TE analysis (fig. 10; but see results of the ML analysis in supplementary data 4.1), our taxon sampling was not designed to test its monophyly. Thus, we transfer *Rhinella ceratophrys* to *Rhaebo* as *Rhaebo ceratophrys* (Boulenger, 1882), new combination.

Rhinella and Its Internal Relationships

In the parsimony total evidence analysis, *Rhinella* was recovered as monophyletic (after transferring *R. ceratophrys* to *Rhaebo*) and well supported (JGC and JAF = 98%). The monophyly of *Rhinella* was previously recovered by several phylogenetic studies that used fewer taxa (e.g., Pauly et al., 2004: fig. 4; Pramuk, 2006; Pyron and Wiens, 2011; Pyron, 2014: suppl. information "amph_shl.tre"; Portik and Papenfuss, 2015; Jetz and Pyron, 2018: suppl. information "amph_shl_new.tre"). In contrast to all previous studies, we found that *Rhinella* is composed of two major, well-supported clades (figs. 11–14; see below).

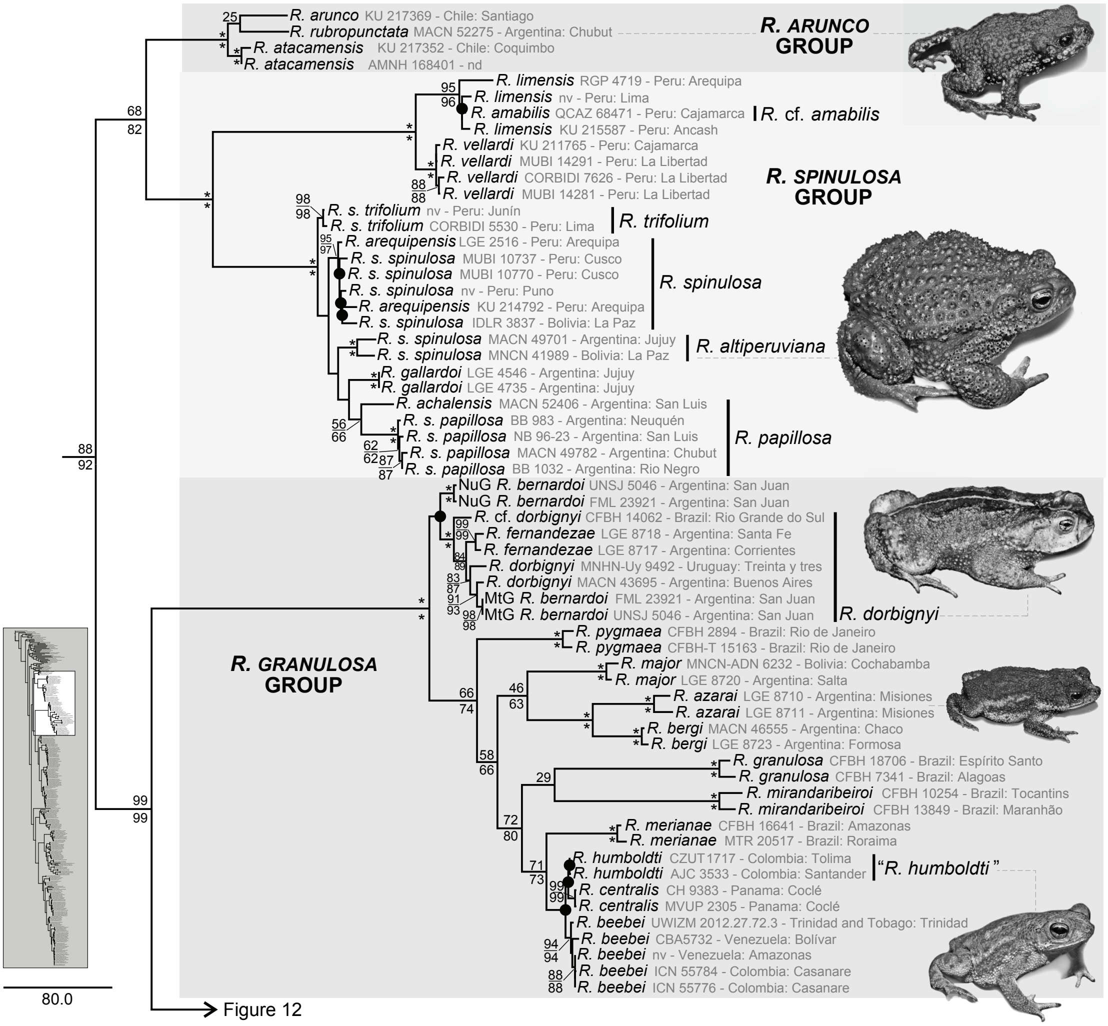

FIG. 11. Phylogenetic relationships of *Rhinella* recovered in one of the most parsimonious trees from the total evidence analysis with TNT considering gaps as a fifth state (length 25,399 steps). The clades and species groups shown are those recognized in this study. Part 1 of 4. The *R. marina* Clade (1): the *R. arunco*, *R. spinulosa*, and *R. granulosa* Groups. Black circles indicate nodes that collapse in the strict consensus. Values around nodes are parsimony jackknife frequencies (frequency differences value [above]/absolute [below]). An asterisk (*) indicates 100% jackknife support. Clades lacking references have <25% frequency difference values or <50% jackknife absolute frequencies. Lower left inset shows the entire cladogram with present view marked in white. Abbreviations: **MtG,** mitochondrial genome; **NuG,** nuclear genome.

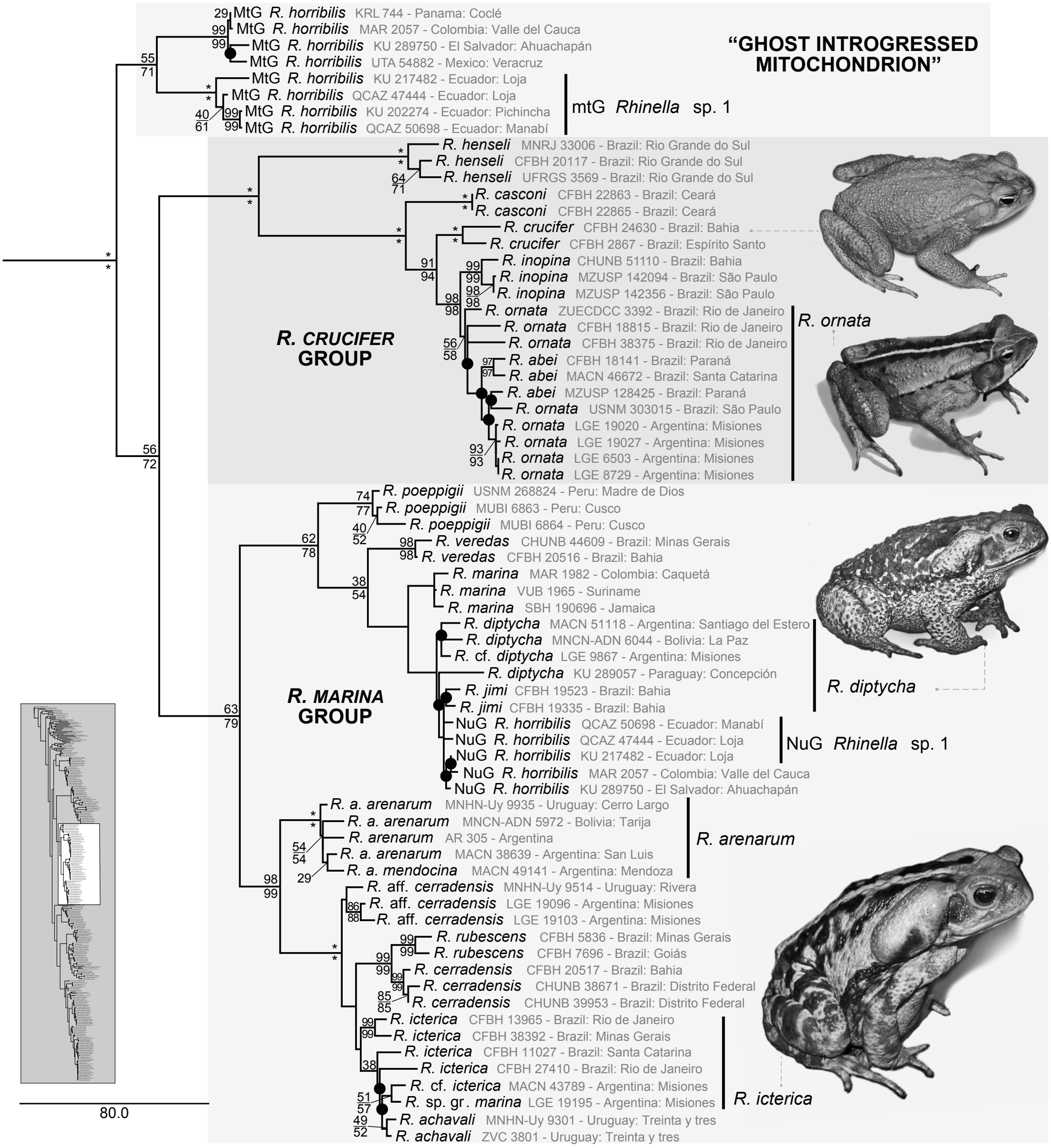

FIG. 12. Phylogenetic relationships of *Rhinella* recovered in one of the most parsimonious trees from the total evidence analysis with TNT considering gaps as a fifth state (length 25,399 steps). The clades and species groups shown are those recognized in this study. Part 2 of 4. The *R. marina* Clade (2): the ghost introgressed mitochondrion and the *R. crucifer* and *R. marina* Groups. Black circles indicate nodes that collapse in the strict consensus. Values around nodes are parsimony jackknife frequencies (frequency differences value [above]/absolute [below]). An asterisk (*) indicates 100% jackknife support. Clades lacking references have <25% frequency difference values or <50% jackknife absolute frequencies. Lower left inset shows the entire cladogram with present view marked in white. Abbreviations: **MtG,** mitochondrial genome; **NuG,** nuclear genome.

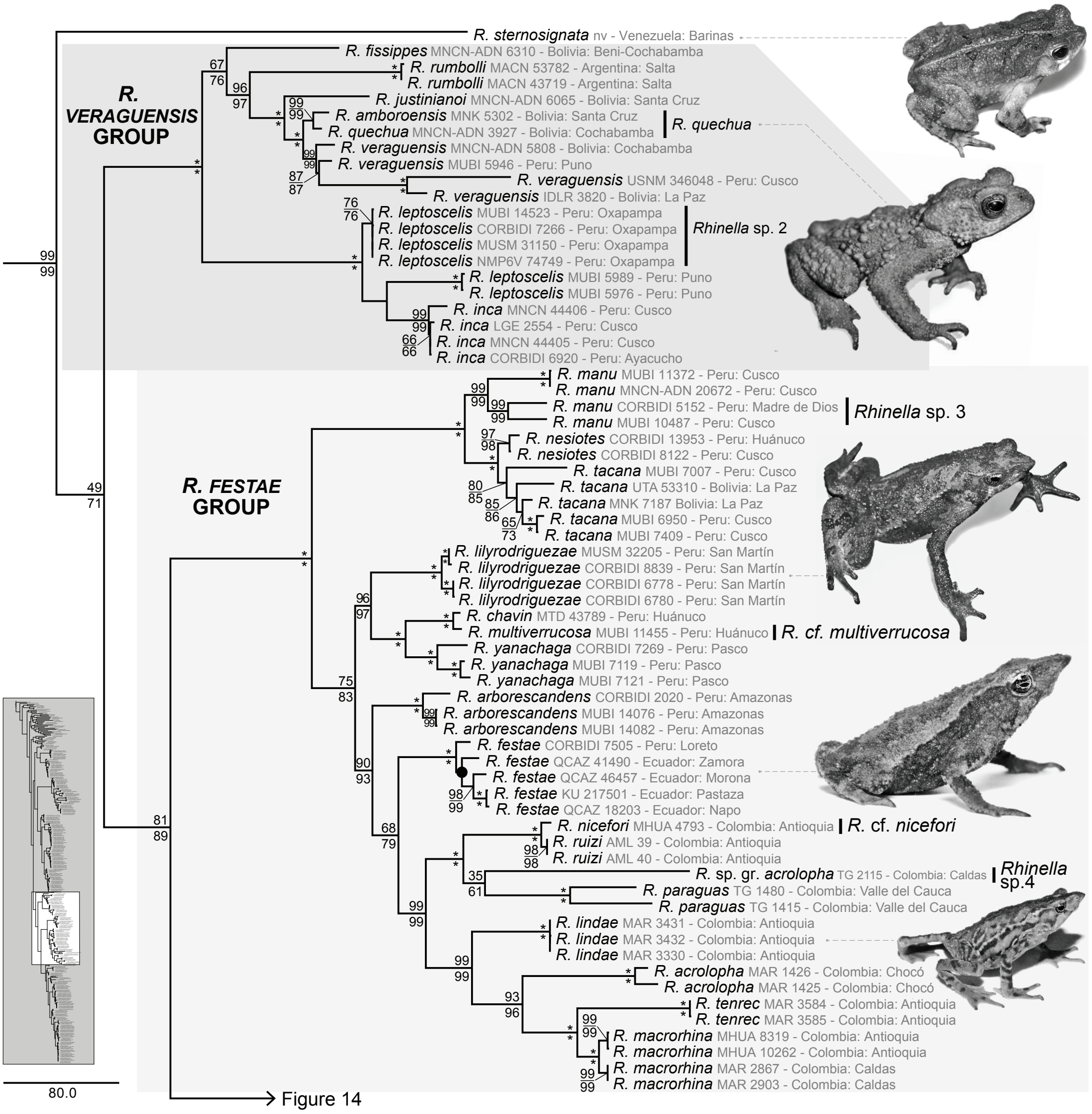

FIG. 13. Phylogenetic relationships of *Rhinella* recovered in one of the most parsimonious trees from the total evidence analysis with TNT considering gaps as a fifth state (length 25,399 steps). The clades and species groups shown are those recognized in this study. Part 3 of 4. The *R. margaritifera* Clade (1): *R. sternosignata* and the *R. veraguensis* and *R. festae* Groups. Black circles indicate nodes that collapse in the strict consensus. Values around nodes are parsimony jackknife frequencies (frequency differences value [above]/absolute [below]). An asterisk (*) indicates 100% jackknife support. Clades lacking references have <25% frequency difference values or <50% jackknife absolute frequencies. Lower left inset shows the entire cladogram with present view marked in white.

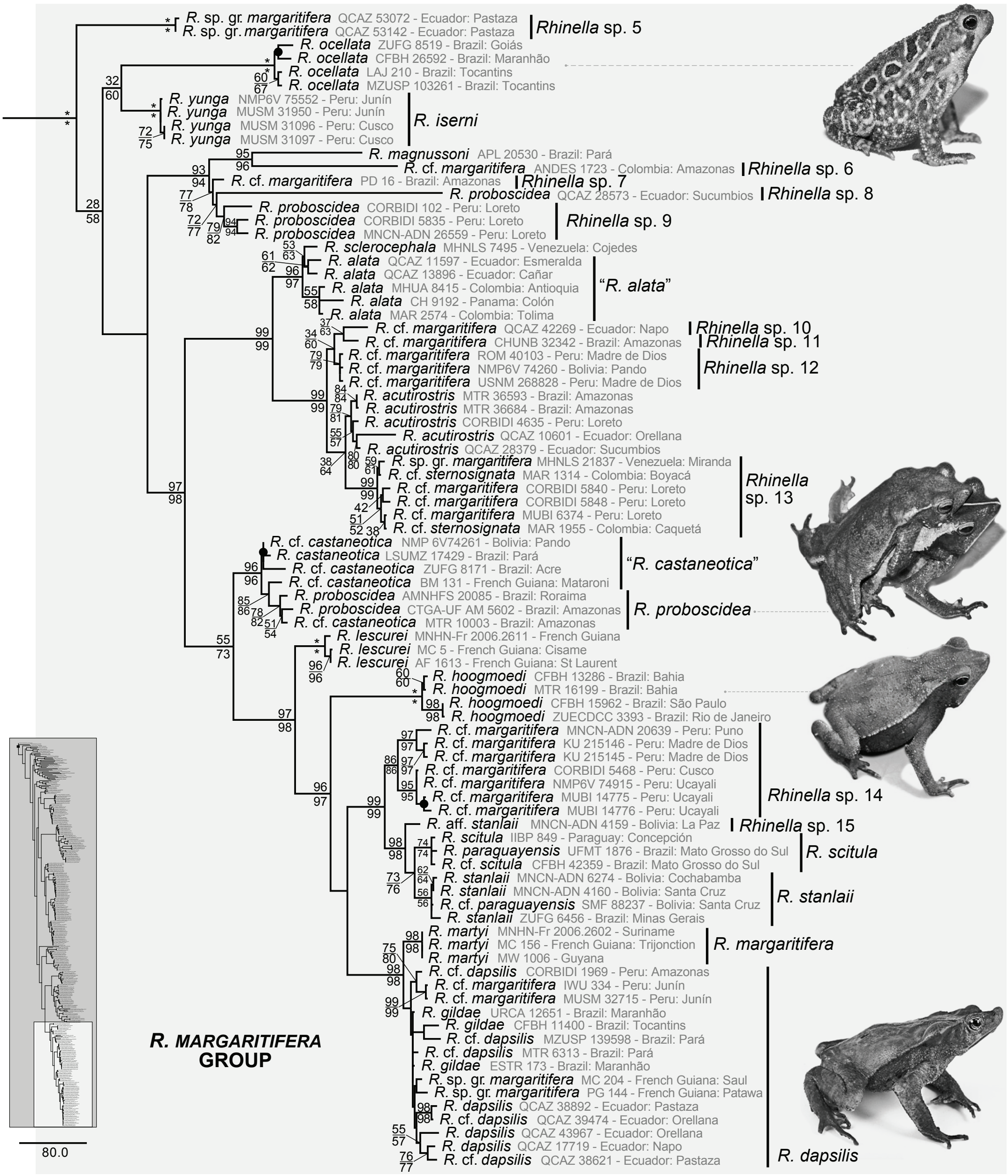

FIG. 14. Phylogenetic relationships of *Rhinella* recovered in one of the most parsimonious trees from the total evidence analysis with TNT considering gaps as a fifth state (length 25,399 steps). The clades and species groups shown are those recognized in this study. Part 4 of 4. The *R. margaritifera* Clade (2): the *R. margaritifera* Group. Black circles indicate nodes that collapse in the strict consensus. Values around nodes are parsimony jackknife frequencies (frequency differences value [above]/ absolute [below]). An asterisk (*) indicates 100% jackknife support. Clades lacking references have <25% frequency difference values or <50% jackknife absolute frequencies. Lower left inset shows the entire cladogram with present view marked in white.

Our results support the *R. crucifer, R. granulosa*, and *R. marina* Groups as monophyletic. Otherwise, the *R. spinulosa* Group is recovered paraphyletic due to the nested position of *R. gallardoi* (a species unassigned to any group). The *R. margaritifera* Group is polyphyletic due to the position of the former *R. ceratophrys* nested in *Rhaebo*. The *R. veraguensis* Group is polyphyletic due to the position of several taxa (i.e., *R. arborescandens, R. chavin, R. lilyrodriguezae, R. manu, R. multiverrucosa, R. nesiotes, R. tacana*, and *R. yanachaga*) more closely related to the *R. margaritifera* Group, and with the monophyletic *R. acrolopha* Group nested within them. The ML analysis of the molecular + phenotypic datasets supported most of these results (supplementary data 4.2–4.5), and we only discuss the relevant differences between analyses. Below, we provide a revised account and comments for *Rhinella* and its main clades and species groups on the basis of these results.

RHINELLA

DIAGNOSIS: The long third pair of external gills (char. 79.2) optimizes as the only phenotypic synapomorphy of *Rhinella* in all the MPTs, which reverts to short third pair of external gills, the plesiomorphic bufonid condition, in the *R. granulosa* Group. An unequivocal diagnosis of this genus is obscured by the large phenotypic variation within *Rhinella* that overlaps with the diversity of many of the related bufonid genera. Nevertheless, this genus can be diagnosed from most of the related bufonids by the combination of the following phenotypic characters: (1) nasals and frontoparietal heavily ornamented with pits, striations, and rugosities (char. 9.2); (2) presence of a row of dorsolateral tubercles on skin (char. 57.1); and (3) nucleolar organizer regions (NORs) located on interstitial position of the short arms of the chromosome pair 7 (char. 89.4).

SISTER CLADE: The well-supported clade composed of *Anaxyrus* + *Incilius* (JGC = 96%, JAF = 97%).

DISTRIBUTION: Mostly Neotropical, ranging from the southern United States to southern South America. *Rhinella marina* is a highly invasive species introduced in many countries and islands outside its native distribution (e.g., Antilles, Australia, Hawaii, Philippines, Taiwan, etc.; see Frost, 2020; IUCN, 2020).

COMMENTS: The exclusion of the former *Bufo ceratophrys* renders *Rhinella* monophyletic. The two large clades of *Rhinella* were not recovered in previous phylogenetic analyses (e.g., Pramuk, 2006: fig. 4; Chaparro et al., 2007; Pramuk et al., 2008; van Bocxlaer et al., 2010: suppl. information S1; Pyron and Wiens, 2011; Pyron, 2014: supp. information "amph_shl.tre"; Portik and Papenfuss, 2015; Pereyra et al., 2016a; Jetz and Pyron, 2018: suppl. information "amph_shl_new.tre"). Vera Candioti et al. (2016) proposed the long third pair of external gills as a putative synapomorphy of *Rhinella* in the context of a review of embryonic morphology of Bufonidae. Our TE analysis supports this character state as synapomorphy of the genus, although the embryonic morphology of many genera of Bufonidae and species of the *R. margaritifera* Clade (see below) is unknown. This synapomorphy of *Rhinella* reverts to the plesiomorphic state (short third pair of external gills) in the *R. granulosa* Group.

As a result of our TE analysis (also see ML result), we define two major clades, the *Rhinella marina* Clade and the *R. margaritifera* Clade, composed of eight species groups within *Rhinella*. The *R. marina* Clade includes (1) the *R. arunco* Group (new species group); (2) the *R. crucifer* Group; (3) the *R. granulosa* Group; (4) the *R. marina* Group; and (5) the *R. spinulosa* Group as redefined here. The second clade, the *R. margaritifera* Clade, is composed of (1) *R. sternosignata*, a species unassigned to any group; (2) the *R. festae* Group as redefined here; (3) the *R. margaritifera* Group as redefined here; and (4) the *R. veraguensis* Group as redefined here. Below, we provide diagnoses, content, and comments on the distribution and systematics of each of the newly defined major clades and all species groups of *Rhinella*. The clades and species group are presented in the order described above and correspond to the sequence in which they appear in the TE tree (figs. 10–14) from base to tip and top to bottom.

THE *RHINELLA MARINA* CLADE (figs. 11, 12)

DIAGNOSIS: This clade is moderately supported (JGC = 88%, JAF = 92%) and diagnosed by a phenotypic synapomorphy: larval otic capsule with poorly developed processus anterolateralis with a rounded aspect (char. 76.1), with one instance of homoplasy in *Sclerophrys regularis*.

SISTER CLADE: The *Rhinella margaritifera* Clade (figs. 13, 14).

CONTENTS: The *Rhinella marina* Clade is composed of the *R. crucifer, R. granulosa*, and *R. marina* Groups, the *R. spinulosa* Group as redefined here, and the *R. arunco* Group, a new group defined here (see below). Moreover, we found a divergent mitochondrial lineage introgressed into *R. horribilis* (hereafter referred to as GIM [ghost introgressed mitochondrion], see below and discussion) that does not seem to belong to any recognized extant species of *Rhinella* and was recovered as sister clade of the *R. marina* + *R. crucifer* Groups (see fig. 12), although with poor support (see below).

DISTRIBUTION: The species of this clade naturally occur in all main biogeographic regions of the Neotropics.

COMMENTS: The *Rhinella marina* Clade is composed of two subclades. One is poorly supported (JGC = 68%, JAF = 82%) and includes the *R. arunco* + *R. spinulosa* Groups (fig. 11). It is diagnosed by four phenotypic synapomorphies: (1) the supraorbital flange on frontoparietal does not extend laterally beyond the lateral margin of the sphenethmoid (char. 17.0, with instances of homoplasy in *R. quechua* and some outgroups); (2) the m. extensor digitorum on the metacarpophalangeal joint of digiti III (char. 44.1, with instances of homoplasy in *Anaxyrus woodhousii* [polymorphic], *Rhinella hoogmoedi, R. jimi,* and *R. rumbolli*); (3) parotoid gland round to ovoid, mostly symmetrical (char. 56.2, with instances of homoplasy in *R. bergi* and several species of the *R. margaritifera* Clade); and (4) vocal sac absent in adult males (char. 58.0, with instances of homoplasy within *Rhinella* and outgroups). The other subclade is well supported (JGC and JAF = 99%) and includes the *R. crucifer, R. granulosa,* and *R. marina* Groups, and the GIM (figs. 11, 12). Three phenotypic synapomorphies are recovered for this subclade: (1) occurrence of a well-developed supraorbital crest (char. 1.2, with instances of homoplasy in several bufonids); (2) occipital artery pathway completely covered with bone (char. 10.2, with instances of homoplasy in bufonids); and (3) general pattern of coloration of caudal musculature of larvae uniformly dark except an unpigmented longitudinal stripe along the inferior edge (char. 69.1, with instances of homoplasy in *R. quechua, R. veraguensis,* and some outgroups).

Previous phylogenetic studies including less complete sampling of *Rhinella* (Pramuk, 2006; Pramuk et al., 2008; van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Portik and Papenfuss, 2015; Pereyra et al., 2016a; Jetz and Pyron, 2018) never found a sister relation between the clade composed of the *R. arunco* + *R. spinulosa* Groups and the clade composed of the *R. granulosa* + (*R. crucifer* + *R. marina*) Groups. Instead, these studies found the *R. arunco* and *R. spinulosa* Groups as: (1) the sister clade of the species of the *R. margaritifera* Clade as defined here (Pramuk, 2006), (2) as sister clade of the remaining species of *Rhinella* (Pramuk et al., 2008; Pereyra et al., 2016a), or (3) as successive sister clades of the remaining species of *Rhinella* (van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Portik and Papenfuss, 2015; Jetz and Pyron, 2018). In contrast to all these analyses, our ML analysis recovers the *R. arunco* Group as the sister clade of the remainder of the *R. marina* Clade, whereas the *R. spinulosa* Group is the sister taxon of the clade composed of *R. granulosa* + (*R. crucifer* + *R. marina*) Groups. This last clade has always been recovered as monophyletic in previous phylogenetic analyses (Pramuk, 2006; van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Portik and Papenfuss, 2015; Pereyra et al., 2016a; Jetz and Pyron, 2018).

THE *RHINELLA ARUNCO* GROUP

DIAGNOSIS: Two phenotypic synapomorphies diagnose this strongly supported (JGC and JAF = 100%) species group: (1) jaw articulation opposite to the fenestra ovalis (char. 16.1, with instances of homoplasy in some species of the *Rhinella granulosa* Group, the *R. margaritifera* Clade, and in *Nannophryne variegata*); and (2) anterior edge of sacral diapophyses perpendicular to the midline axis of the vertebral column (char. 30.1, with instances of homoplasy in *R. crucifer, R. quechua, R. rubescens, R. spinulosa,* and *R. vellardi*). The presence of an insertion of the m. extensor digitorum longus on metatarsophalangeal joint of digit V (char. 40.1) and the presence of an insertion of the m. extensor digitorum on metacarpophalangeal joint of digiti V (char. 45.1) could represent two additional synapomorphies of this group or an internal clade. Moreover, species of the *R. arunco* Group can be distinguished from members of the other species groups of *Rhinella* by the following combination of character states: (1) preorbital crest weak (char. 0.1), (2) occipital artery pathway uncovered with bone (char. 10.0), (3) frontoparietal that does not extend laterally beyond the lateral margin of the sphenethmoid (char. 17.0), (4) medial ramus of the pterygoid fused with the anterolateral margin of the parasphenoid (char. 21.1), (5) m. extensor digitorum longus with an insertion on the metatarsophalangeal joint of the digit IV (char. 39.1), (6) m. extensor digitorum with an insertion on the metacarpophalangeal joint of digiti III (char. 44.1), (7) inguinal fat bodies present (char. 51.1), (8) row of dorsolateral tubercles absent (char. 57.0), (9) vocal sac absent in adult males (char. 58.0), and (10) eggs biserially disposed in strings (char. 87.1).

SISTER CLADE: The *Rhinella spinulosa* Group.

CONTENTS (3 SPECIES): *Rhinella arunco* (Molina, 1782), *R. atacamensis* (Cei, 1962), and *R. rubropunctata* (Guichenot, 1848).

DISTRIBUTION: Species of the *Rhinella arunco* Group are distributed in Argentina and Chile: *Rhinella arunco* and *R. atacamensis* in the Atacama Desert region, *R. rubropunctata* in the Austral Temperate Forest region (Cei, 1962, 1980; Correa et al., 2013). See map 1 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

COMMENTS: Pramuk (2006) found the *Rhinella spinulosa* Group (sensu Duellman and Schulte, 1992) as nonmonophyletic and excluded the species now placed in *Nannophryne* (i.e., *N. apolobambica, N. cophotis, N. corynetes*, and *N. variegata*; see Frost et al., 2006; Frost, 2020). The remaining species constituted a well-supported clade in her combined (morphological and molecular) analysis, being the sister taxon to all the remaining species of *Rhinella*. A subsequent molecular phylogeny (Pramuk et al., 2008) considering a similar taxon sampling and mitochondrial genes, but several different nuclear genes with respect to Pramuk (2006; *cxcr4* and *rag1*-a vs *pomc* and *rag1*-a), recovered this redelimited group as monophyletic with poor support. Previous and subsequent analyses with slightly increased taxon and gene sampling, however, found this group as paraphyletic with respect to all remaining species groups of *Rhinella* (Frost et al., 2006; van Bocxlaer et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Portik and Papenfuss, 2015; Jetz and Pyron, 2018), or as the (poorly supported) sister taxon of all other species of *Rhinella* (Pereyra et al., 2016a). In our TE analysis, the former *R. spinulosa* Group (including *R. gallardoi*, see below) was recovered as monophyletic but poorly supported (JGC = 68%, JAF = 82%). Moreover, the individual monophyly of its sister subclades is strongly supported (both with JGC and JAF = 100%) and can be diagnosed by phenotypic synapomorphies (see Diagnosis of both groups). Our ML analysis found the former *R. spinulosa* Group paraphyletic with respect to the remaining species groups of the *R. marina* Clade (supplementary data 4.2). Based on these observations, we restrict the *R. spinulosa* Group to the strongly supported clade containing most species of the former *R. spinulosa* Group (and including *R. gallardoi*), and exclude the extra-Andean species *R. arunco, R. atacamensis*, and *R.*

rubropunctata that constitute another well-supported clade, herein recognized as the *R. arunco* Group. The southernmost distributed species *R. arunco* and *R. rubropunctata* are recovered as sister taxa, although with poor support (JGC = 25, JAF < 50%). The three species of this group show a high genetic differentiation in comparison to other species groups of the *R. marina* Clade (see tables 3–6). Natural hybridization between *R. arunco* and *R. atacamensis* was reported by Correa et al. (2012, 2013), but they did not find mitochondrial and nuclear introgression outside a narrow hybrid zone.

The *Rhinella spinulosa* Group

Diagnosis: The following character states optimize as phenotypic synapomorphies of this strongly supported group (JGC and JAF = 100%) in our TE analysis: (1) pretympanic crest absent or indistinguible (char. 2.0, with instances of homoplasy in *Rhinella arunco, R. castaneotica, R. festae*, and some outgroups); (2) nasal and frontoparietal bones articulating only laterally (char. 8.1, homoplastic in *R. quechua, R. rubropunctata, R. veraguensis, R. yanachaga, Rhinella* sp. 14, and some outgroups); (3) lightly exostosed dermal roofing bones (char. 9.1, homoplastic in the *R. festae* Group, in several species of the *R. marina* Group, and outgroups); and (4) slightly enlarged otic ramus of squamosal, overlapping with the dorsal surface of the crista parotica (char. 11.1). In addition, species of the *R. spinulosa* Group can be distinguished from members of the other species groups of *Rhinella* by the following combination of character states: (1) occipital artery pathway not covered by bone (char. 10.0), (2) frontoparietal that does not extend laterally beyond the lateral margin of the sphenethmoid (char. 17.0), (3) medial ramus of the pterygoid fused with the anterolateral margin of the parasphenoid (char. 21.1), (4) m. extensor digitorum longus with an insertion on metatarsophalangeal joint of digiti IV (char. 39.1), (5) m. extensor digitorum with an insertion on the metacarpophalangeal joint of digiti III (char. 44.1), (6) inguinal fat bodies present (char. 51.1), (7) multiserial configuration of eggs in the jelly string (char. 87.2), (8) tarsal fold present (char. 65.1), and (9) adhesive gland divided after fusion of the operculum in embryo (char. 82.2).

Sister clade: The *Rhinella arunco* Group.

Contents (9 species): *Rhinella achalensis* (Cei, 1972b), *R. altiperuviana* (Gallardo, 1961) new status, *R. amabilis* (Pramuk and Kadivar, 2003), *R. gallardoi* (Carrizo, 1992), *R. limensis* (Werner, 1901), *R. papillosa* (Philippi, 1902), new status, *R. spinulosa* (Wiegmann, 1834) [including *R. arequipensis* (Vellard, 1959), new synonymy, see below], *R. trifolium* (Tschudi, 1845) new status, and *R. vellardi* (Leviton and Duellman, 1978).

Distribution: This species group is mostly distributed in arid regions along the Andes of Argentina, Bolivia, Ecuador, Chile, and Peru, except *Rhinella gallardoi* that inhabits the humid subandean forest of Argentina (Vellard, 1959; Córdova, 1999; Pramuk and Kadivar, 2003; Lavilla and Cei, 2001). *Rhinella achalensis* and *R. limensis* are the only species of this group with an extra-Andean distribution in the Sierras Pampeanas Centrales in the Pampas region of Argentina and Atacama Desert of Peru respectively (Vellard, 1959; Cei, 1972b). See map 2 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

Comments: The *Rhinella spinulosa* Group as redelimited here is composed of some taxa with controversial taxonomies that are discussed in the context of our results. The widespread, polytypic, and poorly defined species *R. spinulosa* is recovered as nonmonophyletic, with *R. achalensis, R. gallardoi*, and *R. arequipensis* nested within it (fig. 11). Based on our results and considering that "Peru" is the type locality of *R. spinulosa* (and putatively confined to southern Peru, see Vellard, 1959), we restrict the species *R. spinulosa* s.s. to the well-supported lineage (JGC = 95%, JAF = 97%), composed of the populations from southern Peru and northern Bolivia. The lineage containing these populations of *R. spinulosa* also includes the sampled specimen of *R. arequipensis* from Departamento Arequipa, Peru. *Rhinella arequipensis* was originally described as a subspecies of *R. spinulosa* based only on differences in coloration and density of granular formations in the dorsal tegument (Vellard, 1959). Morrison (1992, 1994), Córdova (1999), and Aguilar and Gamarra (2004) did not find morphological, osteological, karyological, or larval differences that could discriminate between *R. spinulosa* and *R. arequipensis*. According to these observations and our results, we consider *Bufo spinulosus arequipensis* Vellard, 1959, a junior synonym of *Rhinella spinulosa* (Wiegmann, 1834). Thus, the species *R. spinulosa* is restricted to the populations distributed mainly along the Andean Puna of Peru and adjacent Bolivia.

Populations of *Rhinella spinulosa* that had been considered as *R. s. trifolium* were recovered as a distinct and strongly supported lineage (JGC and JAF = 98%) sister to a poorly supported clade (JGC <25%, JAF <50%) containing *R. spinulosa* s.s. and several other species of the group (see below). There are several morphological differences between *R. s. trifolium* and *R. spinulosa* s.s. Vellard (1959) pointed out the disposition of the dorsal glands (longitudinal rows in *R. s. trifolium* and a uniform distribution in *R. s. spinulosa*) and the occurrence of a middorsal vertebral line in *R. s. trifolium*, as the main distinguishing characters. Morrison (1992, 1994), Sinsch (1986), Haas (2002), and Pramuk and Kadivar (2003) considered *R. spinulosa* s.s. and *R. s. trifolium* (and also *R. s. flavolineata*) as variations of a single species (see below), although all but Haas failed to provide detailed justification. The morphological comparisons were some superficial and a detailed reevaluation of the specimens and comparisons with topotypes is needed. Córdova (1999) and Aguilar and Gamarra (2004) did not find karyological or larval differences between *R. s. spinulosa* and *R. s. trifolium*; however, these character systems are conserved in related species of *Rhinella* (see Tolledo and Toledo, 2010; Kolenc et al., 2013; Blotto et al., 2014). The UPDs between the specimens of *R. s. trifolium* and *R. spinulosa* s.s. are relatively high for this species group (1.11%–1.30%, see table 4). Consequently, the differences in adult morphology proposed by Vellard (1959) and their genetic divergence support the recognition of *Rhinella trifolium* (Tschudi, 1845) as a distinct species.

Some populations currently assigned to *Rhinella spinulosa* s.l. from Jujuy (Argentina) and La Paz (Bolivia) were recovered as another distinct and strongly supported lineage (JGC and JAF = 100%; see fig. 4) with a low UPD between them (0.18%). In the intermediate area of Puna between these localities (~ 800 km) lays the type locality of *R. s. altiperuviana* (Challapata, Oruro, Bolivia). Gallardo (1961) described this subspecies from two adult females; the characters used to differentiate it from *R. spinulosa* s.l. (i.e., tubercles structure, head shape, tarsal fringe development) show considerable variation, at least, in the studied female specimens from northwestern Argentina. Thus, we tentatively assign these populations to *R. s. altiperuviana*. In addition to the phylogenetic position, these specimens differ in UPDs (see table 4) and adult and larval external morphology (B.L.B., D.B., M.O.P., personal obs.) from other species of the group. For these reasons, these populations should be considered as a distinctive species, *R. altiperuviana* (Gallardo, 1961) from the Andean Puna of Argentina and Bolivia. A detailed taxonomic revision is beyond the scope of this work but will be discussed in a subsequent contribution (B.L.B. and M.O.P., in prep.).

Populations of *Rhinella spinulosa* that had been considered as *R. s. papillosa* are recovered as a strongly supported lineage (JGC and JAF = 100%), sister taxon of *R. achalensis*. Both taxa differ in UPDs (1.10 to 1.47%, see table 4), and are morphologically differentiable from *R. spinulosa* s.s. (B.L.B. and M.O.P., in prep.). Thus, we consider *R. papillosa* (Philippi, 1902), a valid species from the austral Andes of Argentina and Chile.

Rhinella gallardoi is deeply nested within the *R. spinulosa* Group. In the original description, Carrizo (1992) highlighted the "broad skull" of this species over the general morphological similarity with the species of the *R. spinulosa* Group and assigned it to the "*Bufo veraguensis-typho-*

TABLE 3

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella arunco* Group

Values reported are mean (range).

		1	2	3
1	*R. arunco* (N = 1)	—		
2	*R. rubropunctata* (N = 1)	2.87	—	
3	*R. atacamensis* (N = 2)	2.80 (2.67–2.93)	2.41 (2.28–2.55)	0.42

nius" complex. Moreover, *R. gallardoi* is the only species of the *R. spinulosa* Group inhabiting exclusively the Yungas of the Andes in northwestern Argentina.

The specimens of *Rhinella amabilis, R. limensis*, and *R. vellardi* are recovered as a strongly supported clade (JCG and JAF and = 100%), which is the sister taxon of the remaining species of the *R. spinulosa* Group as redefined here. Within this clade, the specimen of *R. amabilis* collapses into a basal polytomy with the specimens of *R. limensis* (the UPDs within the clade composed of these specimens are low [0.19%–0.44%]). *Rhinella amabilis* was differentiated from *R. limensis* by a few characters (development of cranial crests, presence of vocal slits, and shape of the parotoid gland). *Rhinella amabilis* is the only species of the *R. spinulosa* Group distributed north of the Huancabamba depression (Loja, Ecuador), and we could not obtain tissues from this area. The only specimen sampled of this species comes from a locality in the Huancabamba depression region but does not fully correspond with the morphological description of the species. An extensive revision of both species, including topotypical material and comparison with the holotypes is necessary to test the validity of *R. amabilis*.

The currently recognized subspecies *Rhinella spinulosa flavolineata* was not included in our analyses. This subspecies differs from *R. trifolium* only in the conspicuity and time of emergence of the vertebral line. Haas (2002) studied the development of specimens he assigned to the subspecies *R. s. spinulosa, R. s. trifolium*, and *R. s. flavolineata* from the same locality (Mantaro valley, between Concepcion and Huancayo, Junin department, Peru). This author reported that juveniles with variable development (or even absence) of this vertebral line could be obtained from a single clutch, hence, this character seems not to be relevant in differentiating these taxa. The occurrence of *R. spinulosa* s.s. in that locality is debatable (see Vellard, 1959, for comments on the distributions of these taxa) and it is possible that Haas (2002) assigned specimens of *R. trifolium* with poorly defined vertebral line to *R. spinulosa* s.s. (see Haas, 2002: fig. 1). In any case, the results of that study demonstrate that the tempo and level of development of the vertebral line are highly variable. Considering that the different morphs found by this author correspond to intraspecific variation within *R. trifolium*, we consider *Bufo spinulosus flavolineatus* Vellard, 1959, a junior synonym of *Rhinella trifolium* (Tschudi, 1845). *Rhinella trifolium* is considered to inhabit the Central Andean Wet Puna (Vellard, 1959), but additional studies are necessary to determine the precise limits of its geographic distribution and variation with respect to *R. spinulosa* s.s.

The *Rhinella granulosa* Group

Diagnosis: This species group is recovered as monophyletic with strong support (JGC and JAF = 100%) as in previous analyses (Pramuk, 2006; Pereyra et al., 2016a). Four phenotypic

TABLE 4

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella spinulosa* Group

Values reported are mean (range).

		1	2	3	4	5	6	7	8	9
1	*R.* cf. *amabilis* (N = 1)	—								
2	*R. limensis* (N = 2)	0.43 (0.43)	0.19							
3	*R. vellardi* (N = 4)	1.03 (1.03–1.05)	1.06 (0.96–1.16)	0.00 (0.00)						
4	*R. gallardoi* (N = 2)	7.62 (7.62)	6.79 (6.69–6.88)	6.14 (6.12–6.19)	0.00					
5	*R. achalensis* (N = 1)	7.65	6.79 (6.70–6.89)	6.14 (6.12–6.19)	0.19 (0.19)	—				
6	*R. papillosa* (N = 4)	7.44 (7.26–7.65)	6.99 (6.73–7.27)	6.34 (6.16–6.58)	0.81 (0.76–0.95)	0.62 (0.57–0.76)	0.10 (0.00–0.19)			
7	*R. altiperuviana* (N = 2)	7.72 (7.62–7.82)	6.89 (6.69–7.08)	6.43 (6.32–6.58)	0.29 (0.19–0.38)	0.47 (0.38–0.57)	0.71 (0.57–0.95)	0.19		
8	*R. trifolium* (N = 2)	7.73 (7.65–7.81)	6.91 (6.73–7.08)	6.64 (6.54–6.78)	0.76 (0.76–0.77)	0.95 (0.95–0.96)	1.19 (1.14–1.34)	0.48 (0.38–0.57)	0.00	
9	*R. spinulosa* (N = 6)	8.26 (8.04–9.17)	7.35 (7.08–8.14)	6.69 (6.51–7.29)	1.23 (1.14–1.33)	1.42 (1.33–1.52)	1.28 (1.14–1.52)	1.13 (0.95–1.33)	1.23 (1.14–1.33)	0.13 (0.00–0.38)

synapomorphies are recovered for this group: (1) anterior end of the septomaxilla developed (previously considered to be the prenasal bones; see discussion of this character in List and Description of Characters) (char. 14.1); (2) sacral diapophyses with the maximum width greater than its maximum length (char. 29.1), with several instances of homoplasy in *Rhinella* and outgroups; (3) submarginal papillae in the larval oral disc absent (char. 71.0), with instances of homoplasy in several bufonids; and (4) two posterior labial tooth rows in the larval oral disc (char. 72.0), that revert in an internal clade of this group. Moreover, nine additional characters might represent synapomorphies of this group or an internal clade depending on their occurrence in *R. bernardoi* and *R. dorbignyi*, where they are still unknown: (1) anteriorly oriented alary process of the premaxilla (char. 13.2), which also optimizes as a synapomorphy of the *R. margaritifera* Clade and is homoplastic in *Incilius coniferus, Schismaderma carens*, and some species of the *R. marina* Clade; (2) articulation of the zygomatic ramus of the squamosal with the maxilla (char. 15.1), homoplastic in *Peltophryne lemur* and *R. sternosignata*; (3) articulation of the jaw anterior to the fenestra ovalis (char. 16.2), homoplastic in *Melanophryniscus* gr. *stelzneri* and *Peltophryne lemur*; (4) bony sphenethmoid reaching the level of the premaxillae anteriorly (char. 18.2); (5) posterior lobe in the anterolateral process of hyoid absent (char. 25.0), homoplastic in *Rhaebo ceratophrys, Rhinella acrolopha*, and in the *R. margaritifera* Group; (6) vocal sac projected anteriorly when fully inflated (char. 60.1), homoplastic in some species of *Anaxyrus*; (7) short third pair of gills in the embryos (char. 79.1), homoplastic in *Melanophryniscus* gr. *stelzneri* and *Schismaderma carens*; (8) short dorsal line of hatching glands in the embryos (char. 80.0), with an instance of homoplasy in *R. marina*; and (9) type-A adhesive glands in the embryos (char. 81.0).

The species of the *Rhinella granulosa* Group can be distinguished from members of the other groups of *Rhinella* by the following combination of character states: (1) preorbital crest well developed (char. 0.2), (2) supraorbital crest well developed (char. 1.2), (3) pretympanic crest well developed (char. 2.2), (4) nasal and frontoparietal articulate along the entire margin (char. 8.3), (5) occipital artery pathway completely covered with bone (char. 10.2), (6) medial ramus of the pterygoid fused and extending medially along approximately half the length of the parasphenoid alae (char. 21.2), (7) anterior edge of sacral diapophyses perpendicular to the midline axis of the vertebral column (char. 30.1), (8) inguinal fat bodies present (char. 51.1), (9) tarsal fold absent (char. 65.0), (10) caudal musculature of larvae uniformly dark except an unpigmented longitudinal stripe along the inferior edge (char. 70.1), (11) occurrence of irregular transverse whitish stripes in the caudal musculature of larvae (char. 70.1), (12) short third gill pair in the embryo (char. 79.1), and (13) adhesive gland divides immediately before the gills reach their maximum development (char. 82.1).

Sister clade: The clade composed of the GIM (see below) and the *Rhinella crucifer* and *R. marina* Groups.

Contents (13 species): *Rhinella azarai* (Gallardo, 1965); *R. beebei* (Gallardo, 1965); *R. bergi* (Céspedez, 2000); *R. bernardoi* Sanabria et al., 2010; *R. centralis* Narvaes and Rodrigues, 2009; *R. dorbignyi* (Duméril and Bibron, 1841) [including *R. fernandezae* (Gallardo, 1957) new synonymy, see below]; *R. granulosa* (Spix, 1824); *R. humboldti* (Gallardo, 1965); *R. major* (Müller and Hellmich, 1936); *R. merianae* (Gallardo, 1965); *R. mirandaribeiroi* (Gallardo, 1965); *R. nattereri* (Bokermann, 1967); and *R. pygmaea* (Myers and Carvalho, 1952).

Distribution: Species of this group are widely distributed in open areas of Amazonia, Atlantic Forest, Caatinga, Cerrado, Chaco/Pantanal, Chocó, and Pampas regions and in Panama (Narvaes and Rodrigues, 2009; Sanabria et al., 2010; Pereyra et al., 2016a; Murphy et al., 2017). See map 3 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

Comments: In a previous molecular phylogenetic analysis of the *Rhinella granulosa* Group, Pereyra et al. (2016a) recognized 12 putative phenotypic synapomorphies for the group (three of these were first proposed by Pramuk, 2006, and one by Blotto et al., 2014). Eight of these character states were included as part of homology hypotheses (characters) in our TE analysis and only three were corroborated as synapomorphies of the *R. granulosa* Group in all the MPTs (all the remaining were recovered as synapomorphies in some MPTs). The remaining four character states (i.e., the presence of an expanded flag-shaped dorsal crest of the ilium in lateral view; nasal bone articulates with the dorsal margin of the pars facialis of the maxilla from the preorbital process to the posterior margin of the narial opening; occipital condyles widely separated; and ability to build and inhabit holes in the ground) were not included due to the lack of detailed descriptions or preparations for many species of *Rhinella*. However, these character states are unique of the *R. granulosa* Group among the most closely related groups and are consequently considered putative synapomorphies of this group.

Taxonomic, genetic, and biological aspects of the *Rhinella granulosa* Group were addressed in detail by Pereyra et al. (2016a), but some differences need to be stressed. First, we found variations in the inferred relationships among the earlier diverging clades/species of this group. Our TE analysis recovered a basal polytomy that comprises: (1) *R. bernardoi,* (2) *R. dorbignyi* (including *R. fernandezae,* see below), and (3) a poorly supported clade (JGC = 66%, JAF = 74%) composed of the remaining species of the group. Pereyra et al. (2016a) found *R. major* to be the sister species of a poorly supported clade (JAF <50, no JGC value reported) comprising all the remaining species of the group. Although in both analyses the interspecific relationships are poorly supported in general, we presume that these differences are due to the inclusion of phenotypic characters, the inclusion of sequences of *R. humboldti*, the denser outgroup sampling in this study, and the inclusion of a contaminated fragment of cytochrome *b* (KP684992; contaminated with *R. icterica*) in the dataset of Pereyra et al. (2016a).

Pereyra et al. (2016a) retained *Rhinella dorbignyi* and *R. fernandezae* as different species, although they noted the absence of reciprocal monophyly between both taxa and the very low genetic distances among the sampled specimens. Although we did not add additional specimens or sequences to our analyses (but a set of phenotypic characters was added in the TE analysis) and we recovered the same topology as Pereyra et al. (2016a) for the clade containing both taxa, we consider *Bufo granulosus fernandezae* Gallardo, 1957, a junior synonym of *Rhinella dorbignyi* (Duméril and Bibron, 1841). This decision is consistent with the criteria followed to synonymize other taxa of *Rhinella* (i.e., absence of reciprocal monophyly, absence of genetic differentiation, and absence of conspicuous differential morphological characters). Different populations of *R. dorbignyi* s.s. vary only in the level of development of the cranial crest, but not in other phenotypic or molecular characters. We hypothesize that local environmental factors through the area of distribution (i.e., Espinal, Humid Chaco, Humid Pampa, and Uruguayan Savanna) could affect the levels of ossification in the skull, resulting in differential development of cranial crests. The genetic and environmental causes of hyperossification are still not well understood in anurans (Paluh et al., 2020; Blotto et al., 2021). The differential patterns of bone deposition on the skull of *R. dorbignyi* are drastic and generate large morphological differences, making this species an excellent candidate to explore the role and impact of environmental factors on hyperossification.

We recovered *Rhinella humboldti* as distinct from *R. beebei*, as obtained by Murphy et al. (2017). However, both specimens of *R. humboldti* collapse in a polytomy together with the well supported *R. centralis* (JGC and JAF = 99%). Both taxa seem to differ in several morphological characters (Narvaes and Rodrigues, 2009; although these authors considered *R. beebei* and *R. humboldti* as a single taxon) and the UPDs between the specimens of both species are 1.04%–1.37% (see table 5). The poor internal resolution of this clade could be due to the reduced gene sampling for both specimens of *R. humboldti* (see appendix 2). However, a thorough analysis including additional molecular markers and morphological comparisons with *R. humboldti* s.s. is necessary to test the validity of *R. centralis*.

The Mitochondrial Lineage of *Rhinella horribilis*

The included mitochondrial sequences of *Rhinella horribilis* together with the *R. crucifer* + *R. marina* Groups constitute a strongly supported clade (JGC and JAF = 100%) in the TE analysis. Within this clade, they are recovered as sister taxon of a poorly suported clade (JGC = 56%, JAF = 72%) formed by the two aforementioned groups. Alternatively, this lineage is recovered in the ML analysis as the sister of the *R. crucifer* Group, with low support (44% ultrafast bootstrap support value). This clade is, in turn, sister to the *R. marina* Group (supplementary data 4.3). As we discuss below (see "Hybridization and genetic introgression in *Rhinella*"), the strong phylogenetic incongruence between mitochondrial and nuclear sequences of all the sampled specimens of *R. horribilis* is the result of a past hybridization with introgression event in which *R. horribilis* incorporated this mitochondrial lineage and completely replaced the original mtDNA of this species. We hypothesize that this mitochondrial lineage corresponds to a still unknown, or perhaps even extinct species of *Rhinella*, as we could not associate it to any of the 92 included species. In addition, two well-supported lineages are genetically differentiated within this mitochondrial clade according to the tree topology and proportionately large genetic distances (mean UPD = 4.19%, table 6): one includes most populations of *R. horribilis* from Colombia and Central America, which we associate to *R. horribilis* s.s., whereas the second lineage includes populations of *R. horribilis* from Ecuador that represent an undescribed species (*Rhinella* sp. 1). This structure is not recovered by the nuclear sequences of *R. horribilis* because they collapse in polytomy.

The *Rhinella crucifer* Group

Diagnosis: This species group was recovered as monophyletic and well supported (JGC and JAF = 100%), as in previous studies (Maciel et al., 2006; Thomé et al., 2010, 2012). Three phenotypic characters states optimize as synapomorphies of the *Rhinella crucifer* Group: (1) insertion of the m. extensor digitorum longus on the metatarsophalangeal joint of digiti IV absent (char. 39.0), which is homoplastic in a subclade of the *R. granulosa* Group, in the *R. margaritifera* Clade, and in some of the earlier-diverging bufonids; (2) lateral m. dorsometatarsalis proximalis digiti IV with a discrete tendon inserting on the proximal interphalangeal joint of digiti IV (char. 42.1), with an instance of homoplasy in *Nannophryne variegata*; and (3) the occurrence of a vertebral line (char. 54.1), with several instances of homoplasy within *Rhinella*. Other additional character states that could optimize as a synapomorphy of this group or an internal clade, depending on their occurence in *R. casconi* and *R. henseli*, that are still unknown: (1) dorsal protuberance of the illium small, low, and laterally projected (char. 32.1; condition within the group known only in *R. crucifer*); and (2) inguinal fat bodies absent (char. 51.0), with instances of homoplasy in *R. achavali, R. rumbolli,* in a subclade of the *R. festae* Group, in the *R. margaritifera* Group, and in several sampled outgroups.

Species of the *Rhinella crucifer* Group can be distinguished from members of the other groups of *Rhinella* by the following combination of character states: (1) supraorbital crest well developed (char. 1.2), (2) pretympanic crest weak (char. 2.1), (3) nasal and frontoparietal articulate along most of its margin but not completely (char. 8.2), (4) occipital artery pathway completely covered with bone (char. 10.2), (5) medial ramus of the pterygoid fused medially along approximately half the length of the parasphenoid ala (char. 21.2), (6) head of the m. extensor carpi ulnaris

TABLE 5

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella granulosa* Group

Values reported are mean (range).

		1	2	3	4	5	6	7	8	9	10	11
1	*R. dorbignyi* (N = 7)	0.22 (0.00–0.38)										
2	*R. pygmaea* (N = 2)	2.44 (2.29–2.67)	0.19 (0.19)									
3	*R. major* (N = 2)	2.93 (2.68–3.26)	1.62 (1.33–1.91)	0.38 (0.38)								
4	*R. azarai* (N = 2)	2.16 (2.10–2.30)	1.81 (1.71–1.91)	2.10 (1.72–2.49)	0.77							
5	*R. bergi* (N = 2)	2.97 (2.68–3.36)	2.27 (1.90–2.65)	2.80 (2.29–3.36)	1.18 (0.94–1.47)	0.00						
6	*R. granulosa* (N = 2)	3.64 (3.25–4.01)	3.62 (3.43–3.81)	3.82 (3.44–4.20)	3.44 (3.25–3.64)	4.19 (3.82–4.57)	0.38					
7	*R. mirandaribeiroi* (N = 2)	4.88 (4.40–5.48)	5.42 (4.96–5.91)	5.51 (4.95–6.13)	4.36 (4.01–4.74)	5.36 (4.77–5.76)	4.97 (4.58–5.40)	0.71				
8	*R. merianae* (N = 2)	3.89 (3.83–4.03)	3.92 (3.82–4.01)	3.82 (3.63–4.01)	3.64 (3.64)	5.09 (4.60–5.59)	3.83 (3.64–4.02)	5.05 (4.59–5.51)	0.00			
9	"*R. humboldti*" (N = 2)	2.75 (2.67–2.91)	2.69 (2.47–2.92)	2.88 (2.67–3.12)	2.69 (2.48–2.92)	3.66 (3.43–3.88)	2.79 (2.67–3.11)	4.28 (4.01–4.54)	2.70 (2.68–2.72)	0.21		
10	*R. centralis* (N = 2)	3.21 (3.05–3.44)	3.23 (3.04–3.42)	3.33 (3.05–3.62)	3.15 (2.85–3.44)	4.17 (3.81–4.55)	3.71 (3.43–4.00)	4.57 (4.20–4.95)	2.96 (2.86–3.05)	1.19 (1.05–1.33)	0.19	
11	*R. beebei* (N = 5)	3.96 (3.64–4.36)	3.40 (3.05–3.95)	3.49 (3.05–4.15)	3.49 (3.06–3.95)	4.43 (3.63–5.23)	4.45 (4.01–4.76)	5.17 (4.57–5.63)	2.91 (2.68–3.14)	2.16 (2.07–2.29)	2.55 (2.29–2.71)	0.72 (0.00–1.25)

TABLE 6

Percentage of uncorrected p-distances between 16S sequences among terminals of the ghost introgressed mitochondrion

Values reported are mean (range).

		1	2
1	*R. horribilis* (N = 4)	0.89 (0.19–1.34)	
2	*Rhinella* sp. 1 (N = 4)	4.23 (3.24–5.98)	0.49 (0.00–0.86)

from the radioulna with an origin via a flat tendon (char. 47.1), (7) parotoid gland approximately ellipsoid (char. 56.0), (8) tarsal fold present (char. 65.1), (9) caudal musculature of larvae uniformly dark except an unpigmented longitudinal stripe along the inferior edge (char. 69.1), and (10) adhesive gland of the embryo divides after opercular fusion (char. 82.2).

Sister clade: The *Rhinella marina* Group.

Contents (5 species): *Rhinella casconi* Roberto et al., 2014; *R. crucifer* (Wied, 1821); *R. henseli* (Lutz, 1934); *R. inopina* Vaz-Silva et al., 2012; and *R. ornata* (Spix, 1824) [including *R. abei* (Baldissera et al., 2004), new synonymy, see below].

Distribution: These species are distributed mainly along the Atlantic Forest region, except *R. inopina*, which inhabits the Cerrado region (Baldissera et al., 2004; Thomé et al., 2010; Arruda et al., 2014; Roberto et al., 2014). See map 4 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

Comments: The general internal relationships among the species are similar to those reported by Thomé et al. (2010, 2012). Previously, Maciel et al. (2006) recovered this group as monophyletic, but the internal relationships among the species were poorly supported. Based on our results and those of previous analyses, we discuss below several relevant taxonomic issues of this group.

Based on external morphology and morphometric analyses, Baldissera et al. (2004) reviewed the taxonomy of *Rhinella crucifer*. These authors resurrected two species (*R. henseli* and *R. ornata*) and recognized two new species (*R. abei* and *R. pombali*) for several populations previously considered within *R. crucifer.* More recently, two additional species were described, *R. casconi* and *R. inopina* (Vaz-Silva et al., 2012; Roberto et al., 2014). Three of these species (i.e., *R. casconi, R. crucifer*, and *R. henseli)* were recovered as strongly supported lineages (JAF and JGC = 100%), and they have moderate UPDs with respect to other species (>0.98% see table 7).

Thomé et al. (2010, 2012) found *Rhinella abei* nested in *R. ornata* and stressed the need for including additional molecular markers before taking a taxonomic decision on this species. Our analyses, considering additional genes, recovered *R. abei* as nonmonophyletic and nested within *R. ornata*. Moreover, vouchers from multiple localities show no consistent differences in the morphological characters employed by Baldissera et al. (2004) to distinguish these species (e.g., color in preserved specimens, subocular band distinctiveness, head width, and forearm development; M.O.P. and D.B., personal obs.). Thus, we found no evidence to support the distinctiveness of *R. abei*, and consider *Bufo abei* Baldissera et al., 2004, a junior synonym of *Rhinella ornata* (Spix, 1824).

Rhinella ornata (including *R. abei*) is monophyletic, but poorly supported (JGC = 56%, JAF = 58%). Its sister taxon is *R. inopina*, a putatively independent lineage (see FCA analysis in Thomé et al., 2012) recovered with strong support (JGC and JAF = 99%) in the TE analysis. The genetic distances between *R. ornata* and *R. inopina* are very low for the *R. crucifer* Group (0.2%–0.7%) and cannot be attributable to evident mitochondrial introgression (see Thomé et al., 2012; fig. 9); some morphological characters (e.g., adult size, the coloration of marks on flanks, cloacae, and the posterior surface of thighs, and the disposition of parotoid macroglands) were proposed to differentiate both species. Considering the exceptionally low UPDs between *R. ornata* and *R. inopina* and the considerably wide range of *R. ornata*, further comparative studies accounting for geographical variation in these characters are necessary to definitely support or reject the status of *R. inopina* as a distinct species.

The *Rhinella marina* Group

Diagnosis: Our TE analysis recovered a poorly supported *Rhinella marina* Group (JGC = 63%, JAF = 79%) as in previous studies with less dense taxon sampling (e.g., Maciel et al., 2010; van Bocxlaer et al., 2010; Pyron, 2014). Two phenotypic synapomorphies support this species group: (1) the jagged or scalloped articulation between the medial ramus of pterygoid and parasphenoid alae (char. 22.1), with instances of homoplasy in *R. atacamensis, R. achalensis, R. sternosignata*, in a subclade of the *R. festae* Group, and in some species of the *R. margaritifera* Group, and (2) the sacral diapophyses with the anterior edge angled posteriorly to the midline axis of the vertebral column (char. 30.0), with instances of homoplasy in *Rentapia hosii* and *Schismaderma carens*.

Species of the *Rhinella marina* Group can be distinguished from members of the other species groups of *Rhinella* by the following combination of character states: (1) preorbital crest well developed (char. 0.2), (2) supraorbital crest well developed (char. 1.2), (3) pretympanic crest well developed (char. 2.2), (4) nasal and frontoparietal articulate along the entire margin (char. 8.3), (5) occipital artery pathway completely covered with bone (char. 10.2), (6) medial ramus of the pterygoid fused and extending medially along approximately half the length of the parasphenoid ala (char. 21.2), (7) m. extensor digitorum longus with an insertion on the metatarsophalangeal joint of digiti IV (char. 39.1), (8) inguinal fat bodies present (char. 51.1), (9) parotoid gland approximately ellipsoid, longer than wide or triangular and bulky (char. 56.0 or 56.3), (10) tarsal fold present (char. 65.1), (11) adhesive gland division after operculum fusion in embryo (char. 82.2), and (12) eggs biserially disposed in strings (char. 87.1).

Sister clade: The *Rhinella crucifer* Group.

Contents (10 species): *Rhinella achavali* (Maneyro et al., 2004); *R. arenarum* (Hensel, 1867); *R. cerradensis* Maciel et al., 2007; *R. diptycha* (Cope, 1862) [including *R. jimi* (Stevaux, 2002), new synonymy, see below]; *R. horribilis* (Wiegmann, 1833); *R. icterica* (Spix, 1824); *R. marina* (Linnaeus, 1758); *R. poeppigii* (Tschudi, 1845); *R. rubescens* (Lutz, 1925); and *R. veredas* (Brandão et al., 2007).

Distribution: These species are naturally distributed throughout all the main regions of the Neotropics, except in arid Andean areas and the Austral Temperate Forest region (Cei, 1980; De la Riva, 2002; Stevaux, 2002; Kwet et al., 2006; Brandão et al., 2007; Maneyro and Kwet, 2008; Santana et al., 2010; Acevedo et al., 2016; Saito et al., 2016; Venâncio et al., 2017). See map 5 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

Comments: Both recovered phenotypic synapomorphies were suggested as distinctive character states of this group by Pramuk (2006). Moreover, Maciel et al. (2010) proposed four osteological synapomorphies for the *Rhinella marina* Group (ventral ramus of the squamosal ventrolateral in posterior view; anterior extension of the cultriform process extends beyond the orbitonasal foramina; sphenethmoid lightly ossified; medial ramus of the pterygoid relatively narrow) and one skin-secretion compound (occurrence of a specific indolealkylamine). These characters were not considered in our TE analysis and should be reevaluated considering a denser sample of outgroups than the one employed by Maciel et al. (2010).

The finding of a moderately supported *Rhinella marina* Group contrasts with previous studies that recovered it well supported (e.g., Maciel et al., 2010; van Bocxlaer et al., 2010; Pyron, 2014; Jetz and Pyron, 2018). Two distinctive moderately supported clades are evident in

TABLE 7

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella crucifer* Group
Values reported are mean (range).

		1	2	3	4	5
1	*R. henseli* (N = 3)	0.25 (0.00–0.38)				
2	*R. casconi* (N = 2)	3.62 (3.62)	0.00 (0.00)			
3	*R. crucifer* (N = 2)	3.14 (3.04–3.23)	1.43 (1.33–1.52)	0.19 (0.19)		
4	*R. inopina* (N = 3)	3.42 (3.42)	1.33 (1.33)	0.86 (0.76–0.95)	0.25 (0.00–0.38)	
5	*R. ornata* (N = 11)	3.25 (3.04–3.61)	1.30 (1.14–1.52)	0.82 (0.57–1.14)	0.35 (0.19–0.57)	0.29 (0.00–0.76)

this genetically and taxonomically complex species group. The first roughly corresponds to the North-Central Clade of Maciel et al. (2010) and is composed of *R. diptycha* (including *R. jimi*), *R. horribilis, R. marina, R. poeppigii*, and *R. veredas,* but does not include *R. cerradensis* (although see MP tree in Maciel et al., 2010: fig. 3).

Rhinella poeppigii, R. veredas, and *R. marina* s.s. (see Acevedo et al., 2016) are successive sister taxa of the remaining species of the North-Central Clade but their positions are poorly supported (JGC <38%, JAF <54%). Except for *R. veredas*, the monophyly of these species are poorly supported (JGC = 74%, JAF = 77 for *R. poeppigii*; JGC <25%, JAF <50% for *R. marina*). The relationships among *R. diptycha, R. jimi,* and the nuclear sequences of *R. horribilis* and *Rhinella* sp. 1 are poorly resolved (see fig. 12). The lack of resolution and support for the internal relations of the North-Central Clade could be due, at least in part, to (1) the nuclear markers employed that do not provide sufficiently informative variation to resolve the relationships in the absence of mitochondrial information for some terminals, and (2) the occurrence of putative past and present hybridization that could not be detected with the available molecular evidence.

The occurrence of a deep mitochondrial divergence in *Rhinella horribilis* and *Rhinella* sp. 1 precluded the combination of the mitochondrial and nuclear sequences into single terminals. This results in an unstable and poorly supported phylogenetic position for nuclear sequences of both species in the TE analysis (in a polytomy with *R. jimi* specimens). Because the nuclear sequences employed provided relatively few informative characters, their relationships should be better explored considering additional evidence. Moreover, *R. horribilis* diverges in several morphological characters (adult morphology, osteology, and larval morphology; see Savage, 2002; Stevaux, 2002; Kwet et al., 2006; Tolledo and Toledo, 2010; Acevedo et al., 2016) from the largely allopatric *R. jimi*. Otherwise, the position of *R. jimi* in a polytomy together with the specimens of *R. diptycha* and the very low UPDs (0%–0.19%) among the specimens of these species indicate that the taxa are conspecific. *Rhinella diptycha* and *R. jimi* are two morphologically similar species with large parotoid and tibial macroglands. Remarkably, *R. jimi* has distinctive glands on its forearms and on both sides of the cloaca that were considered as the only distinctive characters from *R. diptycha* (Stevaux, 2002; Kwet et al., 2006). Mailho-Fontana et al. (2018) found that both species have similar types and distribution of skin glands, although in different proportions. These authors proposed that this differential development could be related to the occupancy of xeric environments by *R. jimi.* We also found a greater glandular development in the forearms and both sides of the cloaca in some specimens of *R. diptycha* from different localities of the dry Chaco in Argentina (M.O.P. and D.B., personal obs.). Based on these observations, we consider *Bufo jimi* Stevaux, 2002, a junior synonym of *Rhinella diptycha* (Cope, 1862). More physiological and histological studies, investigating different populations from different environments, could help to understand the patterns of variation in the development of macroglands in this species.

The other clade of the *Rhinella marina* Group is composed of *R. achavali, R. arenarum, R. cerradensis, R. icterica*, and *R. rubescens* (fig. 12), and roughly corresponds to the South-Central Clade of Maciel et al. (2010). Within this clade, *R. arenarum* is supported as sister taxon of the remaining species of the clade with strong support (JGC and JAF = 100%). The sampled specimen from the populations that had been considered as *R. arenarum mendocina* is nested within the remaining specimens of *R. arenarum*.

The sister clade of *Rhinella arenarum* is well supported but it is internally poorly resolved. This includes *R. achavali, R. cerradensis, R. icterica*, and *R. rubescens*. *Rhinella cerradensis* and *R. rubescens* are reciprocally monophyletic, their UPDs are low (0.19%–0.74%, see table 8), and constitute a strongly supported clade (JGC and JAF = 99%) that collapses in a basal polytomy within the clade. *Rhinella achavali* was recovered nested in a poorly supported clade (JGC <25%, JAF <50%) composed of some populations of *R. icterica* and the UPDs within this clade are low (0.37%–0.76%, see table 8). Although *R. icterica* is quite variable morphologically (M.O.P. and D.B., personal obs.) and this species includes several synonymized forms (e.g., *Bufo missionum*; Faivovich and Carrizo, 1997), this taxon is divergent morphologically from *R. achavali* (see Maneyro et al., 2004; Kwet et al., 2006; M.O.P., F.K., and C.B., personal. obs.). Finally, some specimens tentatively assigned to *R. cerradensis* (*R.* aff. *cerradensis*) collapse into a basal polytomy within the sister clade of *R. arenarum*. We refrain from taking any decision regarding the taxonomy of these species pending more studies, particularly with respect to understanding the effect of genetic (e.g., nuclear and/or mitochondrial introgressions) and environmental (e.g., phenotypic plasticity) factors on their morphological variation.

The *Rhinella margaritifera* Clade

Diagnosis: This well-supported clade (JGC and JAF = 99%) is diagnosed by two phenotypic synapomorphies: (1) alary process of the premaxillae angled anteriorly to the anterior margin of the pars dentalis of premaxillae (char. 13.2), with instances of homoplasy in *Incilius coniferus, Rhinella achalensis, R. ornata, R. poeppigii*, and *Schismaderma carens*; and (2) skin of dorsum of females with small tubercles lacking cornified tips (char. 52.3).

Sister clade: The *Rhinella marina* Clade.

Contents: *Rhinella sternosignata* and the *R. festae, R. margaritifera*, and *R. veraguensis* Groups.

Distribution: The species of this clade are mainly distributed throughout Amazonia and montane humid forest of the Andes. Some species of this clade are also found in the Atlantic Forest, Caatinga, Cerrado, Chaco/Pantanal, and Chocó regions, and in Central America (Duellman, 1999).

Comments: Within this clade, *Rhinella sternosignata* is recovered as the sister taxon of a large, poorly supported clade (JGC = 49%, JAF = 71%). This last clade is supported by a single phenotypic synapomorphy (ventral ridges on the palatine absent; char. 20.0), which is homoplastic in several species of the *R. marina* Group and outgroups. The clade is composed of three strongly supported species groups (JGC and JAF = 100%): (1) the redefined *R. veraguensis* Group, (2) the redefined *R. festae* Group, and (3) the redefined *R. margaritifera* Group. The *R. festae* and *R. margaritifera* Groups were recovered as sister clades with moderate support (JGC = 81%, JAF = 89%) and five character states optimize as phenotypic synapomorphies of this clade: (1) discrete superficial cutaneous tendons absent (char. 33.0); (2) lateral slip of the m. interphalangeus proximalis digiti V (foot) absent, with instances of homoplasy in *R. major* and *R. papillosa* (char. 35.0); (3) m. abductor brevis plantaris hallucis absent (char. 36.0), with instances of homoplasy in *Anaxyrus woodhousii, Peltophryne empusa* (polymorphic), and *R. mirandaribeiroi*; (4) slip of the medial m. lumbricalis brevis digiti V originating from the distal carpal 3-4-5 absent (char. 43.0) with an instance of homoplasy in *Nannophryne variegata* (polymorphic); and (5) head of the m. extensor carpi ulnaris from the radioulna with a fleshy origin (char. 47.0), with an instance of homoplasy in *P. empusa*. A similar topology for the main internal clades of the *R. margaritifera* Clade was recovered in the ML analysis (supplementary data 4.4–4.5).

Rhinella sternosignata

Diagnosis: *Rhinella sternosignata* (Günther, 1858b) was recovered as the sister taxon of all other species of the *R. margaritifera* Clade, with poor support (JGC = 49%, JAC = 71%). Phenotypic autapomorphies are: (1) acuminate anterior margins of nasals (char. 6.1), with instances of homoplasy in *Incilius coniferus* and the *R. margaritifera* Group; (2) articulation of the zygomatic ramus of the squamosal with the maxilla (char. 15.1), with instances of homoplasy in the *R. granulosa* Group and *Peltophryne lemur*; (3) articulation between the medial ramus of the pterygoid and parasphenoid alae with a jagged suture (char. 22.1) with instances of homoplasy in *R. achalensis, R. atacamensis*, some species of the *R. festae* and *R. margaritifera* Groups, and in the *R. marina* Group; (4) parotoid gland round to ovoid mostly symmetrical (char. 56.2); (5) large size of adult males with respect to adult females (char. 66.1), with instances of homoplasy in *R. yanachaga*, and in several species of the *R. marina* Clade; and (6) unpigmented eggs (char. 88.0), with instances of homoplasy in *Ansonia longidigita, Rhinella justinianoi, R. stanlaii*, and in the *R. festae* Group.

Distribution: This species inhabits montane forests of the Cordillera de la Costa and the Andean Cordillera de Mérida of Venezuela (La Marca and Mijares-Urrutia, 1996; Barrio-Amorós et al., 2019). See map 5 (available at https://doi.org/10.5531/sd.sp.46) for type and sampled localities.

Sister clade: The clade composed of the *Rhinella festae, R. margaritifera*, and *R. veraguensis* Groups.

Comments: This species was tentatively associated with the *Rhinella margaritifera* (Cei, 1972a; Hoogmoed, 1990; Duellman and Schulte, 1992) or *R. granulosa* Groups (Gallardo, 1962). Pereyra et al. (2016a) rejected the inclusion of this species in any of these groups, but they could not determine its relationships rigorously due to the poor sampling of *Rhinella*. This species was wrongly reported for many localities outside the Cordillera de la Costa montane forests region in Venezuela as discussed by La Marca and Mijares-Urrutia (1996). Vélez-Rodríguez (1999) recorded this species in error for Colombia (see Vélez-Rodríguez, 2004b, 2005). Additionally, there are a large number of recent reports of *R. sternosignata* for Colombia (Acosta-Galvis et al., 2006; Romero et al., 2008; Acosta-Galvis, 2012a, 2012b). Analyzed specimens tentatively assigned to this species from the eastern slope of the Cordillera Oriental in Colombia (MAR 1314, Boyacá and MAR 1955, Caquetá) were unrelated to the specimen of *R. sternosignata* from Venezuela in the phylogenetic analyses, and instead, they represent an undescribed species along with other specimens of the *R. margaritifera* Group from Loreto, Peru, and Miranda, Venezuela (*Rhinella* sp. 13, see below). These results, and the absence of comprehensive comparative studies considering topotypical material of *R. sternosignata*, indicate that there is no evidence to consider its occurrence in Colombia.

The *Rhinella veraguensis* Group

Diagnosis: No phenotypic synapomorphies were recovered for this strongly supported group (JGC and JAF = 100%). This is mainly due to the lack of detailed information for one of its two con-

TABLE 8

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella marina* Group

Values reported are mean (range). Species with an asterisk include recent synonyms.

		1	2	3	4	5	6	7	8	9	10
1	*R. poeppigii* (N = 3)	0.25 (0.00–0.38)									
2	*R. veredas* (N = 2)	1.84 (1.71–2.09)	0.00								
3	*R. marina* (N = 3)	2.26 (2.09–2.47)	0.89 (0.76–0.97)	0.26 (0.19–0.39)							
4	*R. diptycha* (N = 7)	2.25 (1.90–2.66)	0.41 (0.19–0.57)	0.42 (0.19–0.59)	0.19 (0.00–0.38)						
5	*R. arenarum* (N = 3)	3.25 (3.04–3.80)	2.13 (2.09–2.28)	2.86 (2.67–3.13)	2.54 (2.28–2.85)	0.15 (0.00–0.38)					
6	*R.* aff. *cerradensis* (N = 3)	3.04 (2.66–3.42)	1.96 (1.71–2.09)	2.60 (2.28–2.86)	2.38 (1.90–2.66)	1.09 (0.76–1.52)	0.25 (0.00–0.38)				
7	*R. rubescens* (N = 2)	2.70 (2.47–3.04)	1.62 (1.52–1.71)	2.34 (2.09–2.54)	2.03 (1.71–2.28)	1.12 (0.95–1.52)	0.54 (0.19–0.76)	0.19			
8	*R. cerradensis* (N = 3)	2.98 (2.66–3.42)	1.90 (1.71–2.09)	2.62 (2.28–2.92)	2.31 (1.90–2.66)	1.41 (1.14–1.90)	0.82 (0.38–1.14)	0.48 (0.19–0.76)	0.38 (0.19–0.57)		
9	"*R. icterica*" (N = 11)	2.92 (2.47–3.42)	1.90 (1.71–2.09)	2.59 (2.09–2.93)	2.31 (1.90–2.66)	1.03 (0.76–1.52)	0.61 (0.19–0.76)	0.73 (0.38–1.14)	1.01 (0.57–1.52)	0.70 (0.38–0.96)	
10	*R. achavali* (N = 2)	2.76 (2.66–3.04)	1.73 (1.71–1.75)	2.45 (2.28–2.55)	2.15 (1.90–2.34)	0.85 (0.76–1.17)	0.64 (0.38–0.78)	0.67 (0.57–0.78)	0.96 (0.76–1.17)	0.58 (0.38–0.78)	0.00

stituent clades (composed of *Rhinella* sp. 2 [see below], *R. inca* and *R. leptoscelis*). Nevertheless, some character states might represent synapomorphies for this group or a subclade: (1) the articulation of jaw opposite to the fenestra ovalis (char. 16.1), with instances of homoplasy in *Nannophryne variegata, Rhinella beebei, R. merianae, R. yanachaga*, and the *R. arunco* Group; (2) light-colored nuptial pads (char. 62.0); (3) larval peribranchial region with bulging regions lateral to the oral disc (char. 67.1); (4) larval oral disc with complete A2 labial tooth row (char. 73.0), with instances of homoplasy in *Amazophrynella* aff. *minuta, Ansonia longidigita, Melanophryniscus* gr. *stelzneri, Phrynoidis juxtaspera*, and *Schismaderma carens*; (5) the dextral opening of the vent tube (char. 75.1); and (6) eggs laid in open clumps (char. 86.1; structure of the spawn only known in *R. rumbolli* within the *R. veraguensis* Group).

The species of the *Rhinella veraguensis* Group can be distinguished from members of the other species groups of *Rhinella* by the following combination of character states: (1) preorbital crest weak (char. 0.1), (2) supraorbital crest weak (char. 1.1), (3) pretympanic crest weak (char. 2.1), (4) medial ramus of the pterygoid fused and extending medially along approximately half the length of the parasphenoid ala (char. 21.2), (5) m. extensor digitorum longus without an insertion on the metatarsophalangeal joint of digiti IV (char. 39.0), and (6) tarsal fold absent (char. 65.0).

SISTER CLADE: The clade composed of the *Rhinella festae* and *R. margaritifera* Groups.

CONTENT (9 SPECIES): *Rhinella chrysophora* (McCranie et al., 1989); *R. fissipes* (Boulenger, 1903); *R. gnustae* (Gallardo, 1967); *R. inca* (Stejneger, 1913); *R. justinianoi* (Harvey and Smith, 1994); *R. leptoscelis* (Boulenger, 1912); *R. quechua* (Gallardo, 1961) [including *R. amboroensis* (Harvey and Smith, 1993), new synonymy, see below]; *R. rumbolli* (Carrizo, 1992); and *R. veraguensis* (Schmidt, 1857).

DISTRIBUTION: All species of the *Rhinella veraguensis* Group are distributed in Andean humid forests of Argentina, Bolivia, and Peru, except *R. chrysophora*, which inhabits the Central American Atlantic moist forests in Honduras (Rodríguez et al., 1993; De la Riva et al., 2000; Köhler, 2000; Lavilla and Cei, 2001; Padial et al., 2009; McCranie, 2017). See map 6 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

COMMENTS: The former *Rhinella veraguensis* Group (see appendix 5) is recovered as polyphyletic. To remedy this, we restrict its content to the clade of species most closely related to *R. veraguensis*. This group also comprises two species not included in the phylogenetic analyses that share several putative synapomorphies with the species sampled here (see below). Two clades were recovered within this redefined *R. veraguensis* Group. One clade is poorly supported (JGC = 67%, JAF = 76%) and comprises the southernmost distributed species of the group. In the second clade, we were unable to examine the voucher of *R. amboroensis* (MNK 5302), but this specimen was collected near the type locality of the species. The specimen was recovered as the sister taxon of *R. quechua* and the genetic distance between the specimens is 0% (see table 9). Both species are very similar morphologically and only a few morphological characters were proposed to differentiate the taxa (i.e., the extension of the foot webbing, ventral skin texture, and finger length). However, these difference are not consistently observed in specimens collected in the type locality of *R. amboroensis* (I.D.L.R., personal obs.) and they could simply represent variations within *R. quechua*. For these reasons, we consider *Bufo amboroensis* Harvey and Smith, 1993, a junior synonym of *Rhinella quechua* (Gallardo, 1961).

The other clade in this group is strongly supported (JGC and JAF = 100%) and includes the northernmost distributed species, *R. inca, R. leptoscelis*, and an undescribed species from Oxapampa, Peru (*Rhinella* sp. 2). The UPDs among these three species are relatively low (1.16%–1.90%; see table 9).

Rhinella chrysophora and *R. gnustae*, two species not included in the phylogenetic analysis, are considered to belong to this species group. *Rhinella chrysophora* is known only from two localities in northern Honduras and is supposedly extinct, not collected since 1996 (McCranie and Castañeda, 2005; McCranie, 2017). This species was originally described as belonging to a distinct genus (*Atelophryniscus*; McCranie et al., 1989) of no evident relationships within Bufonidae. Pramuk and Lehr (2005), based on a morphological phylogenetic analysis, demonstrated that it is related to the species of the *R. veraguensis* Group s.l. Unfortunately, the character scores for *R. chrysophora* are not available and the condition of the double-stained specimen used in that study is very poor (J.J.O.-S., personal obs.). However, morphological evidence indicates that *R. chrysophora* belongs to the *R. veraguensis* Group, as it posses all its known putative synapomorphies (except for oviposition mode, which is unknown; McCranie et al., 1989; Lavilla and de Sá, 2001; Pramuk and Lehr, 2005).

Rhinella gnustae (Gallardo, 1967) was described based on a single subadult specimen from an imprecise locality of Jujuy Province (Argentina) (Gallardo, 1967; Cei, 1980; Lavilla and Cei, 2001; Lavilla et al., 2002). We tentatively assign this species to the *R. veraguensis* Group based on a combination of characters (although none of them synapomorphic) that occur in multiple species of this group: row of dorsolateral tubercles in the skin absent, tarsal fold absent, and small tubercles without a cornified tip.

The *Rhinella festae* Group

Diagnosis: This well-supported group (JGC and JAF = 100%) is diagnosed by the following five phenotypic synapomorphies: (1) skull lightly exostosed (char. 9.1), with instances of homoplasy in *Rhinella achavali, R. rubescens*, the *R. spinulosa* Group, and in several outgroups; (2) fusion of medial ramus of pterygoid with anterolateral margin of the parasphenoid ala (char. 21.1), with instances of homoplasy in *Rhinella* sp. 12, in the *R. arunco* Group, in some species of the *R. spinulosa* Group, and in several outgroups; (3) anterior margin of cultriform process of parasphenoid truncated (char. 23.2); (4) arboreal habits (char. 84.1) that revert in an internal clade of this group, and with instances of homoplasy in *Incilius coniferus* and *Rentapia hosii*; and (5) unpigmented eggs (char. 88.0), with instances of homoplasy in *Ansonia longidigita, Rhinella justinianoi, R. stanlaii*, and *R. sternosignata*. Other putative synapomorphies of this group or an internal clade are: (1) additional origin of the m. interosseus cruris from the tibiale absent (char. 38.0; known within the group only for *R. paraguas*); (2) m. extensor brevis medius hallucis absent (char. 41.0; known within the group only for *R. paraguas*); and (3) the terrestrial oviposition (char. 85.1; known within the group only in *R. tacana*). Moreover, species of the *R. festae* Group can be distinguished from members of the other species groups of *Rhinella* by the following combination of character states: (1) preorbital crest absent or indistinguible (char. 0.0), (2) supraorbital crest weak (char. 1.1), (3) discrete superficial cutaneous tendons absent (char. 33.0), (4) lateral slip of the m. interphalangeus proximalis digiti V absent (char. 35.0), (5) m. abductor brevis plantaris hallucis absent (char. 36.0), (6) m. extensor digitorum longus without an insertion on the metatarsophalangeal joint of digit IV (char. 39.0), (7) slip of the medial m. lumbricalis brevis digiti V originating from the distal carpal 3-4-5 absent (char. 43.0), (8) head from the radioulna of the m. extensor carpi ulnaris with a fleshy origin (char. 47.0), (9) nuptial pads dark colored (char. 62.1), and (10) tarsal fold absent (char. 65.0).

Sister clade: The *Rhinella margaritifera* Group.

Contents (18 Species): *Rhinella acrolopha* (Trueb, 1971); *R. arborescandens* (Duellman and Schulte, 1992); *R. chavin* (Lehr et al., 2001); *R. festae* (Peracca, 1904); *R. lilyrodriguezae* Cusi et al., 2017; *R. lindae* (Rivero and Castaño, 1990); *R. macrorhina* (Trueb, 1971); *R. manu* Chaparro et al., 2007; *R. multiverrucosa* (Lehr et al., 2005); *R. nesiotes* (Duellman and Toft, 1979); *R. nicefori* (Cochran and Goin, 1970); *R. paraguas* Grant and Bolívar-G., 2014; *R. rostrata* (Noble, 1920); *R. ruizi* (Grant, 2000); *R. tacana* (Padial et al., 2006); *R. tenrec* (Lynch and Renjifo, 1990); *R. truebae*

TABLE 9

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella veraguensis* Group

Values reported are mean (range).

		1	2	3	4	5	6	7	8
1	*R. fissipes* (N = 1)	—							
2	*R. rumbolli* (N = 2)	4.56 (4.37–4.75)	0.38						
3	*R. justinianoi* (N = 1)	5.32	4.86 (4.76–4.95)	—					
4	*R. quechua* (N = 2)	3.81 (3.81)	4.19 (3.99–4.38)	3.05 (3.05)	0.00				
5	*R. veraguensis* (N = 4)	4.70 (3.98–5.52)	4.85 (3.80–6.09)	3.85 (3.23–4.38)	1.47 (0.95–2.10)	1.52 (0.95–2.28)			
6	*Rhinella* sp. 2 (N = 4)	7.59 (7.49–7.71)	6.53 (6.31–6.72)	6.35 (6.33–6.37)	6.01 (5.94–6.18)	6.77 (6.49–7.33)	0.10 (0.00–0.19)		
7	*R. leptoscelis* (N = 2)	7.28 (7.28)	5.55 (5.36–5.75)	5.95 (5.95)	5.76 (5.76–5.77)	6.32 (5.93–6.90)	1.78 (1.72–1.93)	0.00	
8	*R. inca* (N = 4)	7.24 (7.15–7.28)	6.71 (6.50–6.95)	6.34 (6.32–6.38)	5.96 (5.94–6.00)	6.71 (6.49–7.15)	1.01 (0.96–1.16)	1.92 (1.91–1.93)	0.00

(Lynch and Renjifo, 1990); and *R. yanachaga* Lehr et al., 2007.

Distribution: Mainly distributed in Andean humid forests of Bolivia, Colombia, Ecuador, and Perú (Trueb, 1971; Duellman and Lynch, 1988; Lynch and Renjifo, 1990; Duellman and Schulte, 1992; Ruiz-Carranza et al., 1996; Lehr et al., 2001, 2005, 2007; Rueda-Almonacid et al., 2004; Chávez et al., 2013; Grant and Bolivar-G., 2014; Cusi et al., 2017). The only species distributed outside this region is *Rhinella acrolopha*, which inhabits the Chocó region (Darién, Panama; Trueb, 1971). See maps 7 and 8 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

Comments: Analyses of our expanded sample of the *Rhinella acrolopha* Group corroborated the monophyly of that group; however, they also corroborated its placement among a subset of the species referred to the *R. veraguensis* Group s.l. Insofar as those species are paraphyletic with respect to the *R. acrolopha* Group, the only means of maintaining the current composition of the *R. acrolopha* Group would be to recognize two or more groups within the larger clade, which we believe to be unwarranted at this time. Consequently, we redefined the *R. festae* Group as was originally proposed by Moravec et al. (2014) to also include all the species previously referred to the *R. acrolopha* Group and three species (*R. arborescandens, R. multiverrucosa*, and *R. tacana*) of the former *R. veraguensis* Group.

Pramuk (2006) proposed a close phylogenetic relationship between *Rhamphophryne* and some species of the *Rhinella veraguensis* Group as formerly defined, but she did not provide a diagnosis for the inclusive clade. As defined in this study, the *R. festae* Group is a morphologically and ecologically diverse clade of toads; the lack of information for many aspects of these species is remarkable (e.g., adult osteology, adult musculature, larval morphology, natural history; see below).

Species of this group have notably high interspecific UPDs compared to species of other groups of *Rhinella* (see tables 3–11) except between the pairs *R. chavin*/*R. multiverrucosa* and *R nicefori*/*R. ruizi*. Sequences of the included specimen assigned to *R. multiverrucosa* (MUBI 11455) are identical (UPD = 0%) to the topotype of *R. chavin* (sequence DQ158441 from Pramuk, 2006). Although morphologically most similar to *R. multiverrucosa*, the specimen MUBI 11455 was not collected near the type locality of this species and was actually collected closer to the type locality of *R. chavin* (see map 8; available at https://doi.org/10.5531/sd.sp.46). Most of the characters that distinguish these two species involve differences in glandular development. Our results should be tested considering the existing morphological variation within *R. chavin* and including topotypes of *R. multiverrucosa* in a future revision of these species.

Similarly, the UPDs between the included specimen of *Rhinella nicefori* and topotypic specimens of *R. ruizi* is low (UPDs = 0.19%; see table 10). The two species were not explicitly differentiated in the original description of *R. ruizi* (Grant, 2000), but they differ in some characters (degree of cranial ornamentation, the occurrence of vocal slits in adult males, adult size). As we did not sample topotypical material of *R. nicefori* and cannot discard the occurrence of some additional variation in the diagnostic characters that differentiate the two species, the identity of the included specimen MHUA 4793 should be reevaluated. For this reason, we refrain from taking a taxonomic action, pending a detailed taxonomic evaluation of both species, considering topotypical material of *R. nicefori* and comparison with type specimens.

Two undescribed species within this species group are recovered in our TE analysis. Firstly, some specimens tentatively assigned to *Rhinella manu* from Madre de Dios and Cusco display high UPDs (3.37%) with respect to specimens of *R. manu* s.s., suggesting they might represent an undescribed species (*Rhinella* sp. 3). Second, the specimen of *R.* sp. "gr. *acrolopha*" (referred to *Rhinella* sp. C. by Machado et al., 2016) from Caldas (Colombia) is recovered as sister species of *R. paraguas*, and the genetic distance between them (UPDs = 5.73%–6.11%) is consistent with the hypothesis that it is an undescribed species (*Rhinella* sp. 4).

We could not include *Rhinella rostrata* and *R. truebae* in our analyses. Nevertheless, these species can be placed in the *R. festae* Group on the basis of several character states that are synapomorphies of this group or its internal clades: (1) skull lightly exostosed (char. 9.1); (2–4) columella, annulus tympanicus, and tympanic membrane absent (chars. 48.0, 49.0, and 50.0) in *R. rostrata* (present in *R. truebae*); and (5) finger webbing present (char. 63.1).

The *Rhinella margaritifera* Group

Diagnosis: No phenotypic synapomorphies were recovered in our TE analysis for this well-supported species group (JGC and JAF = 100%). However, given the lack of information (see Comments on the phenotypic evidence considered for *Rhinella* section) for its earlier diverging species (e.g., *R. ocellata, R. yunga*, and *Rhinella* sp. 5) or closely related clades (i.e., *R. sternosignata*, the *R. festae* and *R. veraguensis* Groups), the inclusion of additional observations in the phenotypic dataset could provide diagnostic synapomorphies for this clade. A putative synapomorphy for this species group (unknown condition in *Rhinella* sp. 5) is the acuminate anterior margins of nasals (char. 6.1), with instances of homoplasy in *Incilius coniferus* and *R. sternosignata*. Moreover, species of the *R. margaritifera* Group can be distinguished from members of the other species groups of *Rhinella* by the following combination of character states: (1) preorbital crest weak (char. 0.1), (2) medial ramus of the pterygoid fused and extending medially along approximately half the length of the parasphenoid ala (char. 21.2), (3) posterior lobe of the anterolateral process of hyoid absent (char. 25.0), (4) discrete superficial cutaneous tendons absent (char. 33.0), (5) lateral slip of the m. interphalangeus proximalis digiti V absent (char. 35.0), (6) m. abductor brevis plantaris hallucis absent (char. 36.0), (7) m. extensor digitorum longus without an insertion on the metatarsophalangeal joint of digiti IV (char. 39.0), (8) slip of the medial m. lumbricalis brevis digiti V originating from the distal carpal 3-4-5 absent (char. 43.0), (9) head of the m. extensor carpi ulnaris from the radioulna with a fleshy origin (char. 47.0), (10) inguinal fat bodies absent (char. 51.0), (11) tarsal fold absent (char. 65.0), and (12) submarginal papillae in the oral disc of larvae absent (char. 71.0).

Sister clade: The *Rhinella festae* Group.

Contents (17 species): *Rhinella acutirostris* (Spix, 1824); *R. alata* (Thominot, 1884); *R. castaneotica* (Caldwell, 1991); *R. cristinae* (Vélez-Rodríguez and Ruiz-Carranza, 2002); *R. dapsilis* (Myers and Carvalho, 1945) [including *R. gildae* Vaz-Silva et al., 2015, new synonymy, see below]; *R. hoogmoedi* Caramaschi and Pombal, 2006; *R. iserni* (Jiménez de la Espada, 1875) [including *R. yunga* Moravec et al., 2014 new synonymy, see below]; *R. lescurei* Fouquet et al., 2007a; *R. magnussoni* Lima et al., 2007; *R. margaritifera* (Laurenti, 1768) [including *R. martyi* Fouquet et al., 2007a, new synonymy, see below]; *R. ocellata* (Günther, 1858b); *R. proboscidea* (Spix, 1824); *R. roqueana* (Melin, 1941); *R. scitula* (Caramaschi and Niemeyer, 2003) [including *R. paraguayensis* Ávila et al., 2010, new synonymy, see below]; *R. sclerocephala* (Mijares-Urrutia and Arends, 2001); *R. sebbeni* Vaz-Silva et al., 2015; and *R. stanlaii* (Lötters and Köhler, 2000).

Distribution: Mainly distributed in Amazonia, but a few species also occur in the Andes, Atlantic Forest, Caatinga, Cerrado, Chocó, Chaco/Pantanal, and in Central America (Hoogmoed, 1986, 1990; Ruiz-Carranza et al., 1996; Caramaschi and Pombal, 2006; Köhler et al., 2006; Fouquet et al., 2007a; Moravec et al., 2014; Sugai et al., 2014; Santos et al., 2015; Ávila et al., 2018; Freitas et al., 2018; Silva et al., 2018). See maps 9 and 10 (available at https://doi.org/10.5531/sd.sp.46) for type localities and sampled localities.

Comments: This species group is particularly controversial regarding its diagnosis, content, and taxonomy of its species. The main revisions

TABLE 10

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella festae* Group

Values reported are mean (range).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*R. manu* (N = 2)	0.00 (0.00)																	
2	*Rhinella* sp. 3 (N = 2)	4.33 (4.01–4.65)	2.52																
3	*R. nesiotes* (N = 2)	4.48 (4.36–4.60)	4.88 (4.56–5.25)	0.81															
4	*R. tacana* (N = 5)	5.49 (5.16–6.12)	5.23 (4.78–5.92)	2.19 (1.72–3.18)	1.64 (0.19–2.86)														
5	*R. lilyrodriguezae* (N = 4)	9.12 (8.92–9.52)	8.21 (7.60–8.91)	8.41 (7.98–8.90)	8.61 (8.15–8.94)	1.35 (0.00–2.13)													
6	*R. chavin* (N = 1)	7.56 (7.55–7.57)	7.28 (6.98–7.58)	7.08 (6.80–7.37)	7.48 (7.36–7.57)	5.55 (5.40–5.80)	—												
7	*R.* cf. *multiverrucosa* (N = 1)	7.47 (7.46–7.48)	7.14 (6.89–7.38)	7.01 (6.75–7.27)	7.37 (7.26–7.45)	5.03 (4.65–5.79)	0.00	—											
8	*R. yanachaga* (N = 3)	6.84 (6.70–6.94)	7.27 (7.08–7.56)	7.37 (6.93–8.11)	7.64 (7.26–8.28)	5.03 (4.65–5.79)	3.63 (3.56–3.77)	3.49 (3.42–3.61)	1.15 (0.19–1.72)										
9	*R. arborescandens* (N = 2)	7.20 (7.08–7.44)	6.92 (6.82–7.02)	7.37 (6.93–8.11)	6.84 (6.52–7.44)	5.36 (5.08–5.82)	5.24 (4.77–6.17)	5.06 (4.59–6.02)	5.32 (4.78–6.19)	1.04 (0.00–1.56)									
10	*R. festae* (N = 5)	7.84 (7.51–8.33)	7.38 (7.09–7.91)	6.90 (6.35–7.64)	7.48 (6.93–8.33)	6.44 (6.01–6.98)	6.17 (5.55–6.74)	6.01 (5.39–6.55)	6.14 (5.57–6.89)	5.16 (4.57–6.23)	1.23 (0.00–1.92)								
11	*R.* cf. *nicefori* (N = 1)	7.96 (7.78–8.05)	8.03 (8.04–8.56)	8.59 (8.35–8.83)	8.81 (8.43–9.39)	6.98 (6.76–7.17)	6.75	6.47	5.86 (5.72–5.95)	5.71 (5.66–5.74)	5.85 (5.72–6.16)	—							

TABLE 10 *continued*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
12	*R. ruizi* (N = 2)	8.15 (8.06–8.24)	8.49 (8.23–8.75)	8.78 (8.55–9.02)	9.01 (8.62–9.58)	7.17 (6.95–7.37)	6.94 (6.94)	6.66 (6.66)	6.05 (5.91–6.14)	5.90 (5.85–5.93)	6.05 (5.91–6.35)	0.19 (0.19)	0.00						
13	*Rhinella* sp. 4 (N = 1)	9.67 (9.59–9.76)	9.45 (9.00–9.91)	8.38 (8.14–8.63)	8.58 (8.24–9.00)	7.70 (7.56–7.94)	7.14	6.85	6.11 (5.90–6.33)	6.47 (6.30–6.81)	6.67 (6.46–6.75)	3.62	3.81 (3.81)	—					
14	*R. paraguas* (N = 2)	8.35 (7.49–9.21)	8.65 (8.24–9.01)	8.64 (7.48–9.80)	8.94 (7.48–10.36)	7.66 (7.36–8.33)	6.85 (6.75–6.95)	6.67 (6.47–6.88)	6.12 (5.34–6.91)	6.73 (6.49–7.00)	7.02 (6.71–7.68)	4.01 (3.81–4.20)	4.20 (4.00–4.39)	5.72 (5.53–5.92)	3.24				
15	*R. lindae* (N = 3)	8.47 (8.42–8.56)	8.38 (8.08–8.73)	8.39 (8.13–8.63)	9.05 (8.75–9.38)	7.23 (6.96–7.65)	6.41 (6.38–6.48)	6.34 (6.31–6.41)	6.34 (6.11–6.62)	5.47 (5.35–5.72)	5.10 (4.79–5.46)	6.15 (6.12–6.21)	6.34 (6.31–6.41)	6.91 (6.87–6.97)	6.60 (6.50–6.70)	0.00 (0.00)			
16	*R. acrolopha* (N = 2)	9.33 (9.24–9.43)	9.28 (9.17–9.39)	9.10 (8.59–9.61)	9.50 (9.21–9.79)	8.23 (7.59–9.31)	7.50 (7.21–7.79)	7.57 (7.29–7.85)	7.15 (6.91–7.53)	6.23 (6.06–6.44)	7.79 (7.16–8.33)	6.42 (6.34–6.51)	6.62 (6.53–6.70)	7.00 (6.92–7.09)	7.49 (7.12–7.86)	6.07 (5.95–6.23)	1.34		
17	*R. tenrec* (N = 2)	9.76 (9.75–9.77)	8.79 (8.62–8.95)	9.84 (9.53–10.15)	10.05 (9.78–10.35)	10.51 (10.27–10.81)	8.57 (8.57)	8.23	9.08 (9.00–9.19)	8.35 (8.24–8.59)	8.24 (7.86–8.57)	9.00 (9.00)	9.19 (9.19)	9.58 (9.58)	9.20 (9.02–9.38)	7.31 (7.27–7.39)	8.63 (8.45–8.82)	0.00	
18	*R. macrorhina* (N = 2)	9.53 (9.20–9.77)	8.72 (8.04–9.31)	7.85 (7.16–8.44)	8.31 (7.87–8.61)	9.00 (8.70–9.46)	8.13 (7.98–8.18)	7.80 (7.66–7.85)	8.13 (7.85–8.41)	6.63 (6.31–7.22)	6.06 (5.36–6.96)	7.90 (7.84–8.05)	8.09 (8.03–8.24)	8.18 (8.05–8.24)	8.10 (7.67–8.44)	6.29 (6.11–6.61)	7.52 (7.09–8.04)	6.37 (6.31–6.52)	1.37 (0.00–2.10)

dealing with this group (e.g., Hoogmoed, 1986, 1990; Duellman and Schulte, 1992; Vélez-Rodríguez, 2004b; Pramuk, 2006; Fouquet et al., 2007a) disagreed with respect to the inclusion of multiple species (e.g., *Rhinella cristinae, R. iserni, R. ocellata*; see appendix 5). Vélez-Rodríguez (2004b) performed a phylogenetic analysis of the group based on morphological characters and proposed the restriction of its content to a clade diagnosed by two synapomorphies: (1) m. depressor mandibulae composed of two slips with independent origins, on the posterior portion of the otic ramus of the squamosal and the anterior portion of the otic ramus of the squamosal and tympanic annulus; and (2) thickening of the ventral margin of the quadratojugal (our char. 24.2). This redefinition of the *R. margaritifera* Group was not supported by the combined (i.e., molecular + morphological characters) phylogenetic analysis of Pramuk (2006). Pramuk (2006) recovered two synapomorphies for the few exemplar species of this group that she included: (1) the expansion of the posterior ramus of the pterygoid and (2) the occurrence of a lateral articulation between the nasals and the preorbital processes of the maxillae (homoplastic). She also found *R. ocellata* to be the sister species of the *R. margaritifera* Group.

Subsequent studies dealing with the taxonomy of this group (e.g., Fouquet et al., 2007a; Ávila-Pires et al., 2010; Lavilla et al., 2013; Vaz-Silva et al., 2015; Avila et al., 2018) did not explicitly follow a definition based on synapomorphies and thus its composition varied. Based on our results, we redefine the *Rhinella margaritifera* Group to include the species listed above that are now grouped exclusively on molecular evidence (although some character states may result in putative synapomorphies, see Diagnosis above). Also, the characters proposed by Vélez-Rodríguez (2004) and Pramuk (2006) should be reevaluated considering relevant species not included in these studies (e.g., *R. sternosignata, R. yunga*, and *Rhinella* sp. 5) to understand their polarity in the context of our results.

The lack of a precise type locality and reference specimens, the large intraspecific (including sexual dimorphism) and interspecific variation in adult size and cranial crest shape and development, and the occurrence of sympatry among some species of the *Rhinella margaritifera* Group turned its taxonomy chaotic and confusing (Hoogmoed, 1989; 1990; Lavilla et al., 2013). A detailed revision of this complex species group is beyond the scope of the present study. As a result, we have been cautious to take taxonomic actions only when evidence is decisive.

The nominal species of the group was described by Laurenti (1768) based on illustrations of Seba (1734) of a specimen from "Brasilia" as the type locality. The identity of this taxon remains unclear after more than two and a half centuries (see discussions in Hoogmoed, 1989; Vélez-Rodríguez, 2004; Fouquet et al., 2007a; Ávila-Pires et al., 2010; Lavilla et al., 2013, 2017). Ávila-Pires et al. (2010) designated the specimen depicted in Seba (1734: pl. 71, figs. 6, 7) as the lectotype of *Rana margaritifera* Laurenti, 1768, and considered the species to be conspecific with *Rhinella martyi* Fouquet et al., 2007a. Subsequently, Lavilla et al. (2013) invalidated the lectotype designation by Ávila-Pires et al. (2010) and, assuming that the type specimen of *R. margaritifera* was lost, designated and described a neotype for this species. More recently, Lavilla et al. (2017) noted that a previous publication (Milto and Barabanov, 2011) had reported the existence of the type of *R. margaritifera*, invalidating the neotype.

Milto and Barabanov (2011) mentioned two specimens (ZISP 257.1 and 257.2) within the type series of *R. margaritifera* without additional comments. Photographs of both specimens are inadequate to determine which one was used in the illustration of Seba (1734; or if both were used) because both are adult females that fully agree with the description and illustrations. Consequently, it is reasonable to consider the specimens found by Milto and Baravanov (2011) to indeed be those used by Seba (1734) and to arbitrarily designate the specimen ZISP 257.1 as lectotype of *Rana margaritifera* Laurenti, 1768.

Additionally, we follow Ávila-Pires et al. (2010) regarding the conspecificity of *Rhinella margaritifera* and *R. martyi* because the lectotype and paralectotype of *R. margaritifera* match almost all the characters used by Fouquet et al. (2007a) to differentiate *R. martyi* from other species of the group (heel extension with hind limbs adpressed and iris coloration unknown in the types of *R. margaritifera*). Thus, we consider *Rhinella martyi* Fouquet et al., 2007a, to be a junior synonym of *R. margaritifera* (Laurenti, 1768).

Having established the identity of *Rhinella margaritifera*, we now introduce our results regarding this species group. An undescribed species from Pastaza (Ecuador), *Rhinella* sp. 5, is recovered with low support (JGC = 28%, JAF = 58%) as the sister taxon of all other species of the clade. The sister group of next most inclusive clade is poorly supported (JGC = 32%, JAF = 60%) and composed of two morphologically and geographically divergent species, *R. yunga* and *R. ocellata*. *Rhinella yunga* was recently described from the montane forest of the Selva Central, Peru. Distinctive characters used to diagnose this species in the original description are also present in the poorly known *R. iserni* (skin of dorsum mostly smooth, degree of development of cranial crest, and especially the absence of all the structures of the tympanic middle ear; Jiménez de la Espada, 1875; Moravec et al., 2014; Hoogmoed, personal commun.; J.M. and M.O.P., personal obs.). In addition to their morphological resemblance, both species were described from nearby type localities from the Peruvian Yungas region. Thus, we consider *Rhinella yunga* Moravec et al., 2014, to be a junior synonym of *Rhinella iserni* (Jiménez de la Espada, 1875).

Rhinella magnussoni, R. cf. *margaritifera* from Amazonas (Colombia), specimens of "*R. proboscidea*" from Ecuador and Peru, and an undetermined specimen of the *R. margaritifera* Group from São Pedro (Amazonas, Brazil) compose a well-supported clade (JGC = 93%, JAF = 94%). *Rhinella magnussoni* and *R.* cf. *margaritifera* from Amazonas (Colombia) have a relatively high UPD (2.10%), which seem to support the specific distinctiveness of the latter (*Rhinella* sp. 6). The results of the phylogenetic analysis (see fig. 14) and UPDs among clades (2.60%–10.27%; see table 11) strongly suggest that the specimen from São Pedro and both populations of "*R. proboscidea*" from Ecuador (Sucumbios) and Peru (Loreto) correspond to three undescribed species (*Rhinella* sp. 7–9). The similarity of these undescribed taxa with the phylogenetically distantly *R. proboscidea* s.s. and *R. castaneotica* (see above) indicates the need for a thorough revision of the "*R. proboscidea*" complex.

Another well-supported clade (JGC and JAF = 99%) includes *Rhinella acutirostris, R. alata, R. sclerocephala, R. sternosignata* from Colombia, and several apparently undescribed species (see below). A nonmonophyletic *Rhinella alata* (sensu Santos et al., 2015) and *R. sclerocephala* constitute a well-supported subclade within this clade (JGC = 96%, JAF = 97%). The latter species was recovered as sister taxon of the Ecuadorian populations of *R. alata* with poor support (JGC = 61% JAF = 62%), and their UPDs are 1.31%–1.52%. Otherwise, the populations of Panama and Colombia were recovered as a poorly supported lineage (JGC = 55%, JAF = 58%) with UPDs of 0.56%–1.52% with respect to its sister clade. Santos et al. (2015) did not find any morphological character or evident genetic differentiation between the populations from Panama and those from Ecuador as to consider them as different taxa. Otherwise, *R. sclerocephala* was differentiated from *R. alata* by several characters such as its larger size, presence of well-developed cranial crests, vertebral apophysis, and bony knob (Mijares-Urrutia and Arends, 2001; Santos et al., 2015), although these characters vary extensively within species of the *R. margaritifera* Group and might be associated with particular environmental conditions over their areas of distribution (see Kutrup et al., 2006; Bandeira et al., 2016). The absence of evident differences between specimens of both clades of *R. alata*, the nested position of *R. sclerocephala* within this clade, and the low UPDs seem to support their conspecificity, but we refrain from synonymyzing both taxa due to the low support for the nested position of the specimen of *R. sclerocephala* and the several morphological differences. Nevertheless, it is evident that a detailed taxonomic revision of both taxa considering additional specimens and molecular evidence is required to better resolve the taxonomy within this clade.

The other subclade includes *Rhinella acutirostris* and four undescribed species (see also ML topology in supplementary data 4.5): *Rhinella* sp. 10 from Napo (Ecuador), *Rhinella* sp. 11 from Amazonas (Brazil), *Rhinella* sp. 12 from Pando (Bolivia) and Madre de Dios (Peru), and *Rhinella* sp. 13 from some localities of Colombia (Boyacá and Caquetá), Peru (Loreto), and Venezuela (Miranda). This latter new species was previously recorded from Colombia as *R. sternosignata* (M.R., personal obs.; see comments for *R. sternosignata*).

The nonmonophyletic *Rhinella castaneotica* and *R. proboscidea* compose a well-supported clade (JGC and JAF = 96%). The internal relationships among the included specimens are poorly resolved and the UPDs are 0.19%–2.75%. Within this clade, we could not obtain samples from the type locality of *R. castaneotica* ("near Cachoeira Jaruá, Rio Xingu, Pará, Brazil"), but we included sequences from a relatively close locality (300 km airline distance) that could represent *R. castaneotica* s.s. (see Fouquet et al., 2012a: fig. S6). We also included sequences of specimens from Manaus (Amazonas, Brazil) and Floresta (Roraima, Brazil) that could be morphologically assigned to *R. proboscidea.* However, the imprecise type locality of this species is "flumen Solimoens (= Rio Solimões)," which comprises the Brazilian section of the Amazon River between the triple border of Brazil-Colombia-Peru and the city of Manaus and has an extension of approximately 1700 km (Vanzolini, 1981). Although we consider that phylogenetic evidence, UPDs (see table 11), and absence of distinctive adult and larval differential characters (see comparisons provided by Caldwell, 1991, and Menin et al., 2006) support their conspecificity, we continue recognizing both taxa pending a thorough revision, including additional samples and detailed comparison with the type material.

The sister taxon of the clade including the problematic *Rhinella castaneotica* and *R. proboscidea*, is well supported (JGC = 97%, JAF = 98%) and includes two successively diverging species (*R. lescurei* and *R. hoogmoedi*), and two subclades. One of these is well supported (JGC and JAF = 99%) and composed of *R. paraguayensis, R. scitula, R. stanlaii*, and two undescribed species: *Rhinella* sp. 14 from SE Peru ("*Bufo* sp. 6" sensu Vélez-Rodríguez, 2004b, and "*Bufo* cf. *margaritifer* 5" sensu Pramuk, 2006), and *Rhinella* sp. 15 from La Paz, Bolivia (which corresponds to *Bufo* sp. 1 of Lötters and Köhler, 2000). The included specimen of *R. paraguayensis* was recovered in a polytomy with the specimens of *R. scitula*; the UPDs among these specimens are low (0.13%–0.3% in the complete the 16S rRNA gene). These parapatric species were differentiated mainly by adult size, crest development, and skin texture. All these characters have been demonstrated to be subject to variation due to specific environmental conditions throughout the distribution of some bufonids (see Kutrup et al., 2006; Bandeira et al., 2016). Thus, we consider *Rhinella paraguayensis* Ávila et al., 2010, to be a junior synonym of *R. scitula* (Caramaschi and Niemeyer, 2003).

The other subclade is also well supported (JGC and JAF = 98%) and includes *Rhinella margaritifera* s.s. and a poorly supported clade (JGC <25%, JAF <50%) composed of *R. dapsilis, R.* cf. *dapsilis, R. gildae*, and several divergent lineages of *R. margaritifera* s.l. (e.g., the lineages called *Rhinella* sp. A and *Rhinella* sp. B by Fouquet et al., 2007c). The clade includes specimens that vary in the degree of development of bony protrusions and cranial crests, dorsal coloration, occurrence of a developed proboscis, and dorsal skin texture (Myers and Carvalho, 1945; Dixon, 1976; Rodríguez and Duellman, 1994; Vaz-Silva et al., 2015; M.O.P., personal obs.). The UPDs within this clade are 0%–2.79% (mean UPD = 1.29%, see table 11). Thus, the absence of unequivocal morphological differences and very low genetic distances indicate that *Rhinella gildae* Vaz-Silva et al., 2015, is a junior synonym of *R. dapsilis* (Myers and Carvalho, 1945).

We could not obtain tissue samples of *Rhinella cristinae* to test its relationships. However, this species can be assigned to this group on the basis of the occurrence of the only putative phenotypic synapomorphy of the group (acuminate anterior margins of nasals; char. 6.1) and a combination of characters typical of this group: (1) preorbital crest weak (char. 0.1), (2) medial ramus of the pterygoid fused and extending medially along approximately half the length of the parasphenoid ala (char. 21.2), (3) posterior lobe of the anterolateral process of hyoid absent (char. 25.0), (4) inguinal fat bodies absent (char. 51.0), and (5) tarsal fold absent (char. 65.0).

Hybridization, Deep Mitochondrial Divergence, and "Ghost Introgression" in *Rhinella*

Reports on natural and artificial hybridization are well known in many bufonids including multiple species of *Rhinella* (e.g., Blair, 1972; Green, 1996; Gergus et al., 1999; Malmos et al., 2001; Masta et al., 2002; Baldo and Basso, 2004; Yamazaki et al., 2008; Goebel et al., 2009; Fontenot et al., 2011; Correa et al., 2012, 2013; Pereyra et al., 2016a; Betto-Colliard et al., 2018). Explosive breeding events with intense male competition for mates and passive female choice (i.e., scramble competition; see Wells, 2007; Pereyra et al., 2016b) is common in many species of several genera of Bufonidae, and premating isolating mechanisms seem to be insufficient to avoid interspecific amplexus in these species (see Blair, 1958; Guerra et al., 2011). Malone and Fontenot (2008) also demonstrated that bufonids require a substantial genetic divergence to achieve postzygotic reproductive isolation. Under this scenario, the common occurrence of hybridization in this family is not surprising.

A particular situation of natural hybridization could happen in "*Rhinella pombali*" (Thomé et al., 2010, 2012), where all individuals of this taxon are considered hybrids between *R. crucifer* and *R. ornata* (Thomé et al., 2010, 2012) and our results are in agreement with this idea. The two included specimens of "*R. pombali*" in preliminary analyses were not recovered as monophyletic in the nuclear analysis and each of them has a unique mitochondrion (one from *R. crucifer* and the other from *R. ornata*). Moreover, available evidence is insufficient to test whether "*R. pombali*" could represent a species of hybrid origin (see Avise, 2008; Darras and Aron, 2015, Lavanchy and Schwander, 2019) and more detailed studies are necessary to explore this possibility.

Although we deliberately excluded the hybrid specimens from our analyses, the impact of natural hybridization in Bufonidae could be currently underestimated due to the difficulties in recognizing hybrids and/or past hybridization events. Introgressive hybridization (both nuclear and mitochondrial) could have an impact on bufonid evolution allowing a faster accumulation of genetic novelties than through mutation alone. The incorporation of additional genetic diversity could impact the acquisition of adaptive phenotypic traits and have a significant role in speciation as is common in diverse taxonomic groups (for reviews see Baack and Rieseberg, 2007; Schwenk et al., 2008; Toews and Brelsford, 2012; Abbott et al., 2016; Gopalakrishnan et al., 2018; Hill, 2019; Servedio and Hermisson, 2019).

Mitochondrial introgressions are more commonly reported than are nuclear introgressions and can be evidenced by genetic populational studies or by the discordance between phylogenetic trees inferred from separate analyses of both genomes (Toews and Brelsford, 2012; Bonnet et al., 2017). Within *Rhinella*, putative events of mitochondrial introgression were documented for *R. marina* (Sequeira et al., 2011; but also see Vallinoto et al., 2017 and Bessa-Silva et al., 2020), *R. bernardoi* (Pereyra et al., 2016a), and *R. horribilis* s.l. (present study), and they occur without noticeable evidence of nuclear introgression, as was also reported in other vertebrates (Alves et al., 2006; Chen et al., 2009; Schwarzer et al., 2012).

TABLE 11

Percentage of uncorrected p-distances between 16S sequences among species of the *Rhinella margaritifera* Group
Values reported are mean (range).

		1	2	3	4	5	6	7	8	9	10	11	12
1	*Rhinella* sp. 5 (N = 2)	0.00											
2	*R. ocellata* (N = 4)	7.58 (6.71–8.99)	0.49 (0.00–0.96)										
3	*R. iserni* (N = 4)	5.34 (5.29–5.39)	4.63 (4.23–5.27)	0.00 (0.00)									
4	*R. magnussoni* (N = 1)	6.12 (6.08–6.16)	6.11 (5.75–6.83)	3.24 (3.24)	—								
5	*Rhinella* sp. 6 (N = 1)	5.34 (5.29–5.39)	4.95 (4.62–5.58)	3.44 (3.44)	2.10	—							
6	*Rhinella* sp. 7 (N = 1)	6.95 (6.91–6.98)	7.97 (7.24–9.86)	4.58 (4.58)	3.50	3.97	—						
7	*Rhinella* sp. 8 (N = 1)	13.45 (13.42–13.49)	15.55 (15.04–16.39)	12.25 (12.25)	11.67	11.65	9.66	—					
8	*Rhinella* sp. 9 (N = 3)	5.87 (5.69–6.01)	6.88 (6.40–8.04)	4.17 (3.85–4.39)	3.58 (3.25–3.82)	3.33 (3.26–3.44)	2.77 (2.61–2.90)	9.93 (9.63–10.29)	0.66 (0.58–0.80)				
9	*R. sclerocephala* (N = 1)	7.81 (7.80–7.82)	8.64 (7.99–9.84)	5.34 (5.34)	5.13	6.46	6.94	14.95	7.20 (6.85–7.51)	—			
10	"*R. alata*" (N = 5)	5.75 (6.68–6.94)	6.99 (8.42–)	3.98 (3.68–4.21)	4.48 (4.20–4.78)	4.90 (4.63–5.15)	6.11 (5.60–6.42)	14.01 (13.86–14.19)	5.91 (5.31–6.30)	1.41 (1.28–1.50)	0.96 (0.57–1.35)		
11	*Rhinella* sp. 10 (N = 1)	7.11 (7.07–7.14)	5.76 (5.24–6.78)	4.45 (4.45)	5.21	4.45	6.90	14.69	6.09 (6.01–6.17)	3.82	3.21 (2.90–3.67)	—	
12	*Rhinella* sp. 11 (N = 1)	7.41 (7.28–7.53)	6.12 (5.57–7.03)	4.78 (4.78)	5.93	4.97	7.24	15.17	6.40 (6.30–6.49)	4.46	3.63 (3.28–4.00)	0.78	—
13	*Rhinella* sp. 12 (N = 3)	7.90 (7.07–9.31)	6.14 (5.19–7.08)	4.95 (4.40–5.83)	6.56 (5.92–7.63)	5.41 (4.79–6.43)	8.40 (7.26–10.42)	15.87 (15.17–17.06)	7.00 (6.40–8.09)	4.64 (4.25–5.19)	3.74 (2.90–5.14)	1.36 (1.16–1.55)	1.68 (1.53–1.79)
14	*R. acutirostris* (N = 5)	7.31 (6.90–7.73)	7.09 (5.97–8.46)	4.99 (4.62–5.37)	6.20 (5.76–6.51)	5.83 (5.56–6.14)	8.00 (7.58–8.42)	15.43 (15.02–15.58)	7.04 (6.62–7.35)	4.28 (4.11–4.52)	3.72 (3.26–)	2.12 (1.93–2.31)	2.88 (2.68–3.07)
15	*Rhinella* sp. 13 (N = 6)	6.81 (6.69–7.09)	6.55 (5.59–7.92)	5.45 (5.19–5.83)	6.39 (6.12–6.76)	5.51 (5.18–5.82)	8.07 (7.78–8.57)	15.58 (15.38–15.82)	7.29 (6.98–7.72)	4.77 (4.55–5.20)	4.13 (3.63–4.85)	2.83 (2.51–3.09)	3.58 (3.26–3.88)
16	"*R. castaneotica*" (N = 4)	6.46 (5.69–7.87)	5.81 (4.79–7.21)	3.47 (2.87–4.86)	4.59 (4.01–5.92)	4.33 (3.83–5.45)	5.66 (4.64–7.68)	13.42 (12.65–14.94)	4.80 (3.86–6.12)	4.61 (4.08–5.60)	3.47 (2.70–4.97)	3.69 (3.28–4.35)	4.02 (3.65–4.60)
17	*R. proboscidea* (N = 3)	6.94 (6.08–8.61)	6.53 (5.18–8.21)	4.11 (3.25–5.33)	4.98 (4.21–6.38)	4.79 (3.83–6.20)	6.36 (4.81–7.93)	13.91 (13.25–14.90)	5.64 (4.47–7.12)	4.69 (4.06–5.53)	3.79 (2.70–4.91)	4.05 (3.09–5.11)	4.46 (3.45–5.37)
18	*R. lescurei* (N = 3)	7.57 (6.47–8.41)	6.05 (4.80–6.91)	5.79 (4.77–6.46)	6.03 (5.15–6.50)	5.44 (4.59–6.02)	8.67 (6.77–9.93)	14.50 (13.58–15.17)	6.51 (5.61–7.41)	6.53 (5.74–6.95)	5.26 (4.39–5.76)	5.72 (4.82–6.19)	5.99 (5.16–6.44)
19	*R. hoogmoedi* (N = 4)	7.03 (6.48–8.33)	6.33 (5.38–7.40)	4.78 (4.40–5.57)	4.93 (4.59–5.61)	4.81 (4.40–5.67)	7.10 (6.40–8.60)	13.99 (13.65–14.63)	6.25 (5.50–7.39)	5.34 (4.95–6.09)	4.24 (3.82–5.17)	4.98 (4.45–5.87)	5.26 (4.79–6.12)
20	*Rhinella* sp. 14 (N = 7)	6.32 (6.09–6.57)	5.14 (4.22–6.52)	4.06 (3.83–4.40)	4.46 (4.21–4.83)	4.11 (4.01–4.27)	5.90 (5.72–6.25)	13.35 (13.03–13.42)	5.71 (5.10–6.18)	4.97 (4.70–5.19)	3.74 (3.43–4.25)	3.83 (3.47–4.06)	4.18 (3.81–4.45)
21	*Rhinella* sp. 15 (N = 1)	5.93 (5.89–5.97)	5.86 (5.18–6.78)	3.82 (3.82)	4.39	4.02	5.95	13.04	5.35 (5.09–5.53)	4.71	3.48 (3.44–3.63)	3.47	3.82
22	*R. scitula* (N = 2)	6.12 (6.09–6.16)	6.48 (5.76–7.55)	4.01 (4.01)	4.58 (4.58)	4.21 (4.21)	6.16 (6.16)	13.23 (13.23)	5.54 (5.29–5.72)	4.71 (4.71)	3.56 (3.44–3.63)	3.86 (3.86)	4.20 (4.20)
23	*R. stanlaii* (N = 4)	6.01 (5.71–6.16)	5.98 (5.19–6.99)	3.88 (3.64–4.01)	4.50 (4.40–4.58)	4.17 (4.08–4.21)	5.98 (5.17–6.14)	12.81 (12.71–12.84)	5.41 (4.88–5.72)	4.64 (4.50–4.69)	3.52 (3.29–3.63)	3.43 (3.33–3.47)	3.78 (3.68–3.82)
24	*R. margaritifera* (N = 3)	5.99 (4.91–6.52)	6.22 (4.99–7.13)	4.63 (3.82–5.04)	4.01 (3.44–4.30)	4.47 (3.63–4.89)	5.74 (4.63–6.30)	12.77 (12.25–13.03)	5.14 (4.29–5.83)	5.70 (4.95–6.07)	4.45 (3.63–4.91)	5.85 (4.82–6.37)	6.13 (5.16–6.62)
25	*R. dapsilis* (N = 15)	6.64 (5.69–8.59)	6.04 (4.80–7.42)	4.10 (3.63–5.06)	4.74 (4.01–5.88)	4.91 (4.09–6.20)	5.99 (5.10–7.68)	13.40 (12.18–14.35)	5.77 (4.90–7.12)	5.10 (4.30–6.40)	3.93 (3.05–5.19)	4.92 (4.07–6.40)	5.21 (4.27–6.65)

TABLE 11 *continued*

		13	14	15	16	17	18	19	20	21	22	23	24	24
13	*Rhinella* sp. 12 (N = 3)	0.06 (0.00–0.19)												
14	*R. acutirostris* (N = 5)	2.86 (2.29–3.66)	0.69 (0.19–1.15)											
15	*Rhinella* sp. 13 (N = 6)	3.63 (2.86–4.70)	1.67 (1.34–2.13)	0.49 (0.00–0.77)										
16	"*R. castaneotica*" (N = 4)	4.40 (3.64–5.34)	4.85 (4.23–5.79)	5.09 (4.23–6.58)	1.19 (0.19–2.28)									
17	*R. proboscidea* (N = 3)	4.91 (3.44–5.90)	4.99 (4.02–6.26)	5.20 (4.03–6.80)	1.82 (0.76–2.83)	1.31 (0.98–1.78)								
18	*R. lescurei* (N = 3)	5.99 (4.77–6.46)	6.50 (5.35–7.09)	6.09 (4.96–7.10)	4.16 (3.24–4.90)	4.80 (3.82–5.20)	0.85 (0.00–1.28)							
19	*R. hoogmoedi* (N = 4)	5.80 (4.79–6.68)	5.56 (4.97–6.77)	5.31 (4.60–6.78)	3.30 (2.57–4.43)	3.92 (3.25–4.38)	2.90 (2.49–3.17)	0.46 (0.00–0.79)						
20	*Rhinella* sp. 14 (N = 7)	4.90 (4.20–6.15)	4.46 (4.02–5.04)	4.12 (3.63–4.46)	3.51 (2.29–4.91)	3.77 (2.87–4.86)	3.48 (2.48–4.14)	2.39 (1.53–2.82)	0.57 (0.00–0.97)					
21	*Rhinella* sp. 15 (N = 1)	4.76 (4.20–5.65)	4.55 (4.40–4.78)	4.66 (4.40–5.03)	2.91 (2.29–4.39)	3.28 (2.86–3.82)	3.83 (3.25–4.14)	2.48 (2.29–2.78)	1.50 (1.15–1.74)	—				
22	*R. scitula* (N = 2)	4.75 (4.20–5.65)	4.55 (4.40–4.78)	4.66 (4.40–5.02)	3.01 (2.48–4.40)	3.22 (2.87–3.84)	3.61 (3.06–3.90)	2.28 (2.10–2.54)	2.24 (1.72–2.51)	1.15 (1.15)	0.00 (0.00)			
23	*R. stanlaii* (N = 4)	4.71 (4.07–5.62)	4.53 (4.28–4.79)	4.62 (4.28–5.04)	3.25 (2.71–4.37)	3.48 (2.72–4.33)	3.82 (3.05–4.36)	2.65 (2.33–3.04)	1.79 (1.55–2.33)	1.01 (0.78–1.34)	0.96 (0.78–1.15)	0.42 (0.00–0.77)		
24	*R. margaritifera* (N = 3)	6.54 (5.15–6.94)	6.63 (5.36–7.27)	6.37 (4.97–7.27)	4.00 (2.86–5.20)	4.63 (3.44–5.15)	4.07 (3.25–4.27)	3.04 (2.48–3.33)	2.83 (2.10–3.32)	3.49 (2.86–3.80)	3.72 (3.05–4.05)	3.54 (2.68–4.02)	0.00 (0.00)	
25	*R. dapsilis* (N = 15)	5.80 (4.58–7.10)	5.67 (4.78–7.29)	5.65 (4.78–7.54)	3.60 (2.29–6.04)	4.31 (3.25–5.47)	3.45 (2.48–4.50)	2.49 (1.72–3.42)	2.72 (1.53–3.88)	2.90 (2.29–3.56)	2.98 (2.29–3.84)	3.19 (2.30–4.33)	2.31 (1.33–3.14)	1.22 (0.00–2.60)

Several populations of *Rhinella marina* from south of the Amazon River seem to have similar mitochondrial lineages as *R. diptycha*, in contrast to populations northward. As nuclear loci of specimens of both populations of *R. marina* were similar, and divergent from *R. diptycha*, the occurrence of an extensive mtDNA unidirectional introgression from *R. diptycha* into *R. marina* was hypothesized (Sequeira et al., 2011). However, this hypothesis was not conclusively corroborated in a subsequent study because an additional mitochondrial clade, found for some populations of *R. marina*, obscured the direction of the introgression between these species (Vallinoto et al., 2017). A similar situation of possible unidirectional mitochondrial introgression from *R. dorbignyi* to *R. bernardoi* was reported by Pereyra et al. (2016a). Evidence that supports this hypothesis comes from the well-supported incongruence between the independent analyses of the mitochondrial and nuclear genes: *R. bernardoi* is deeply nested within *R. dorbignyi* in the mitochondrial analysis, but not in the nuclear analysis.

Our results from independent mitochondrial and nuclear analyses (rMD and rND, respectively) also show incongruence in the position of the specimens of *Rhinella horribilis* s.l. We recover this species deeply nested within (morphologically similar) species of the *R. marina* Group in the rND analysis, whereas in the rMD analysis it is recovered as sister of all the species of the *R. crucifer* + *R. marina* Groups. Another striking characteristic of this case of hybridization is the origin of these mitochondria, which is not traceable to any known extant species. These particular forms of deep mitochondrial divergence were denominated “ghost introgressions” (see Zhang et al., 2019). This kind of event involving deep mitochondrial divergence that implies past mitochondrial introgression from an unknown and not closely related species is uncommon in anurans. Historical interspecific introgressions events were reported in several groups of Anura: *Ameerega* (Dendrobatidae; Brown and Twomey, 2009); *Anaxyrus*, *Bufo*, and *Bufotes* (Bufonidae; Malmos et al., 2001; Yamazaki et al., 2008; Colliard et al., 2010; Dufresnes et al., 2019); *Bombina* (Bombinatoridae; Hofman and Szymura, 2007; De Cahsan et al., 2019); *Dyscophus* (Microhylidae; Orozco-terWengel et al., 2013); *Hyla* (Hylidae; Lamb and Avise, 1986; Bryson et al., 2010, 2014; Klymus et al., 2010); *Mantella* (Mantellidae; Crottini et al., 2019); *Pelophylax* and *Rana* (Ranidae; Liu et al., 2010; Zhou et al., 2012; Eto et al., 2013); *Quasipaa* (Dicroglossidae; Zhang et al., 2018); and *Scutiger* (Megophryidae; Chen et al., 2009). However, most of these events (except in *Bombina*, *Bufotes, Quasipaa*, and *Scutiger*) occurred among closely related species. Another striking characteristic of this phenomenon in *Rhinella horribilis* s.l. is that after the ancient introgression, the GIM (i.e., the mitochondrial DNA) diversified into two divergent clades (UPDs >3.33%). We consider most plausible the hypothesis that these mitochondrial clades represent two different species (*R. horribilis* s.s. and *Rhinella* sp. 1) that are not fully detectable (e.g., recovered as monophyletic) with our limited nuclear dataset. More intense genomic and phylogeographic sampling will be necessary to eventually solve the taxonomic status and puzzling history of *R. horribilis* and its lineages.

The reports of hybridization and mitochondrial introgression in *Rhinella* suggest the need for an extensive and careful exploration of these phenomena in other lineages of Bufonidae. The particular reproductive biology (i.e., scramble competition), the occurrence of broad sympatric areas between related species, and genetic features (i.e., complete reproductive isolation with relatively high levels of genetic divergence; Malone and Fontenot, 2008) of many bufonids may facilitate the occurrence of these phenomena. The identification of foreign mitochondrial genomes is particularly relevant to avoid errors both in phylogeographic and taxonomic studies (especially DNA barcoding studies) and phylogenetic inferences (Ballard and Whitlock, 2004; Alves et al., 2006; Obertegger et al., 2018; Barley et al., 2019). Moreover, the identification of mitochondrial introgressions will serve, among other things, as a base for future studies on adaptive coevolution between these organelles and the nuclear components of the oxidative metabolism of the cell (Hill, 2019).

Comments on the Phenotypic Evidence Considered for *Rhinella*

Our phenotypic sampling results in some synapomorphic and/or diagnostic characters for several internal clades of *Rhinella*, including most of the species groups. However, an evaluation of the available information for the character systems used clearly shows large gaps in the knowledge within each species group/clades (see fig. 15).

In general, there is relatively more information available for species of the *Rhinella marina* Clade. Within the *R. margaritifera* Clade, characters on adult osteology and musculature, natural history, and larval morphology are poorly known, and characters of larval chondrocranium, cytogenetics, and embryonic morphology are virtually unknown. This is a source of ambiguity in the reconstruction of ancestral character states for many characters that optimize in more inclusive nodes (e.g., oviposition mode within the *R. festae* and *R. veraguensis* Groups).

With the exceptions of foot and hand musculature, external larval, and embryonic morphologies described for several species of *Rhinella* (e.g., Mercês et al., 2009; Tolledo and Toledo, 2010; Blotto et al., 2014; Vera Candioti et al., 2016, 2020; Grosso et al., 2020; B.L.B., personal obs.), detailed descriptions considering ontogenetic variation, sexual dimorphism, and interpopulation variations are still largely necessary. It must be noticed that these and many other character systems are promising as additional sources of evidence to be included in future phylogenetic analyses. Some examples of variation within species of *Rhinella* were reported on bioacoustics (W.F. Martin, 1972; De la Riva et al., 1996; Guerra et al., 2011; Andrade et al., 2015; Valencia-Zuleta et al., 2020); integument and parotoid macroglands structure (O'Donohoe et al., 2019); anatomy of urogenital and digestive systems (Stohler, 1932; Lynch and Renjifo, 1990; but see Grant, 2000); clutch and egg size (Liedtke et al., 2014; Pereyra et al., 2015); mandibular, pelvic, and thigh musculature (Noble, 1922; Limeses, 1964, 1965; Trueb, 1971; Winokur and Hillyard, 1992; Grant and Bolívar-G., 2014); and secretions (Cei et al., 1968; Maciel et al., 2006; Rodríguez et al., 2017). An inclusive sampling considering all these characters will contribute to the study of patterns of evolution of different character systems and their functional and adaptive significance.

The Fossil Record of *Rhinella* and Calibration Points

As is common for most neobatrachian anuran families, the pre-Pleistocene fossil record of Bufonidae is deficient, and most of these specimens lack an apomorphy-based diagnosis to unambiguously assign them to particular nodes or species (see Parham et al., 2012). The currently known pre-Pleistocene fossils of *Rhinella* are phylogenetically concentrated within the *R. marina* Group: (1) *R. arenarum* (as *R. pisanoi*) from Pliocene outcrops (3.9–3.2 Ma) of coastal Buenos Aires Province, Argentina (Casamiquela, 1967; Pérez-Ben et al., 2014); (2) *R. loba*, an extinct species putatively related to *R. arenarum*, from the late Pliocene (4.5–3.2 Ma) from the Chapadmalal Formation of Argentina (Pérez-Ben et al., 2019); (3) *R. marina* from the mid Miocene (13.8–11.8 Ma) from La Venta fauna of Colombia (Estes and Wassersug, 1963); and (4) *R.* aff. *arenarum* and *Rhinella* sp. *marina* Group from the upper Oligocene (29–26 Ma) of Salla, Bolivia (Báez and Nicoli, 2004). Another fossil from the upper Paleocene (59.2–56 Ma) from Itaboraí, Brazil (Estes, 1970) was also considered as related to some of the South American species groups of the former *Bufo*, but all proposed associations are vague and tentative (see Estes, 1970; Estes and Reig, 1973; Báez and Gasparini, 1977); even an association with *Rhinella* is controversial. Only the Miocene specimen of *R. marina* has been used as a calibration point (along with few other non-*Rhinella* bufonid fossils) in divergence dating analyses of Bufonidae or its inter-

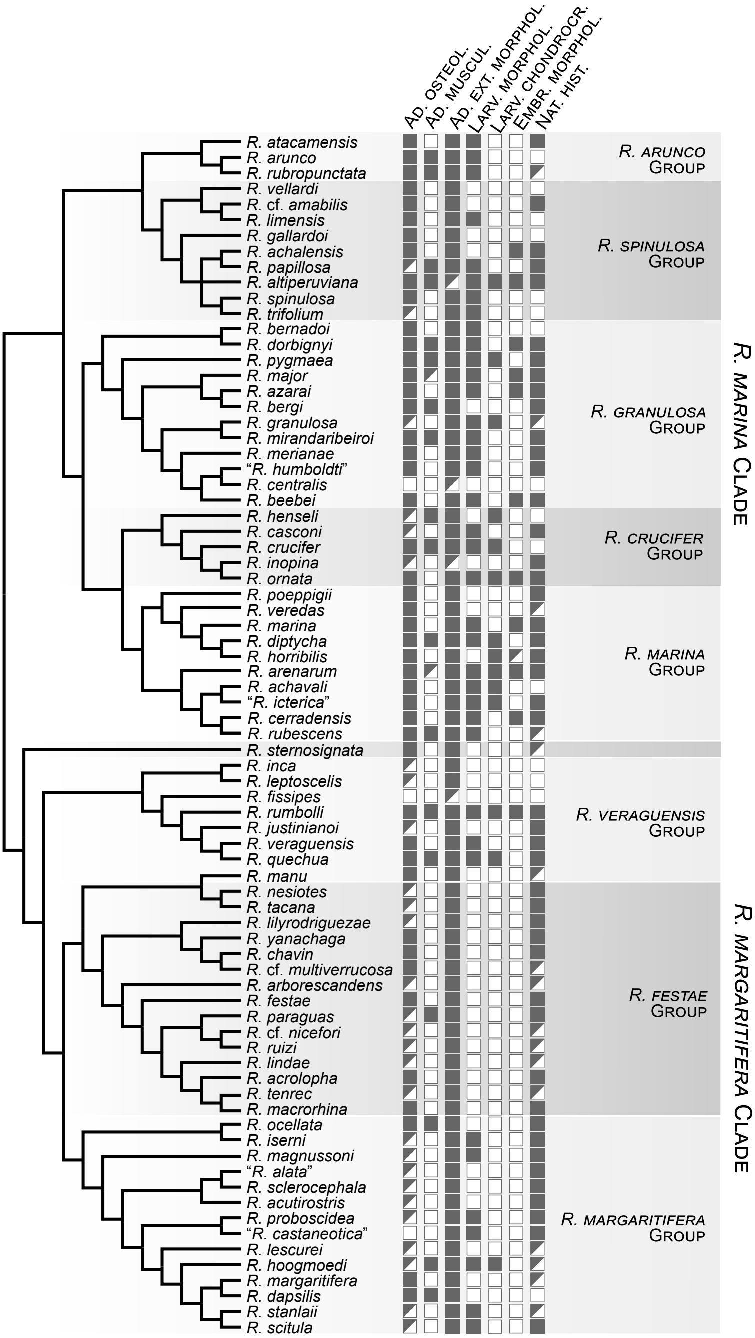

FIG. 15. Main character systems scored for each included species of *Rhinella*. References: Filled squares, most characters of the considered character system scored (> 50%); half-filled squares, most characters not scored (<40%); empty squares, no character scored. Abbreviations: Ad. ext. morphol., Adult external morphology; Ad. musc., Adult musculature; Ad. osteol., Adult osteology; Emb. morphol., Embryonic morphology; Larv. chondrocr., Larval chondrocranium; Larv. morphol., Larval external morphology; Nat. hist., Natural history.

nal clades (e.g., Pramuk et al., 2008; Maciel et al., 2010; van Bocxlaer et al., 2010; Liedtke et al., 2016, 2017). These studies differ in the sampled taxa and genes, and their results are not fully congruent, but the divergence-time estimates, considering relaxed molecular clocks and similar calibration points, indicate a split time between *Rhinella* and *Anaxyrus* + *Incilius* around 41 Ma (34–47 Ma; Pramuk et al., 2008) and 38.7 Ma (28.5–51.8; van Bocxlaer et al., 2010). However, the absence of an understanding of the interspecific osteological variation in species of *Rhinella* and the absence of an apomorphy-based determination of the fossils could result in the association of fossils to a lower-level taxon than the data can demonstrate (see Bever, 2005; Parham et al., 2012). Consequently, a critical reexamination of the available pre-Pleistocene fossils of *Rhinella*, along with an extensive study of living species of all the species groups is necessary before their defensible use as calibration points in divergence dating analyses. If material from Itaboraí can be unambiguously associated with *Rhinella*, its inclusion will provide a crucial point of calibration that could modify extensively our current understanding of the patterns of diversification of *Rhinella* and also of Bufonidae.

CONCLUSIONS

Our results provide a general framework for better understanding the evolution and taxonomy of *Rhinella* and its internal clades. The main results of our work include: (1) the generation of a well-supported phylogenetic hypothesis of the genus resulting from a total evidence analysis of most of its specific diversity, (2) the redefinition and morphological diagnosis of its species groups, (3) the demonstration of hybridization and mitochondrial introgression between some species, and (4) evaluation of the taxonomic status of several species. Nevertheless, many challenges are still pending. For example: (1) the taxonomic revision of many clades, including the designation of neotypes for several taxa; (2) the evaluation of the ontogenetic and intersexual variation in several problematic taxa; (3) the use of denser gene sampling (with high throughput sequencing) to better understand the evolutionary relationships in poorly supported clades and evaluate the role of the introgressive hybridization in the evolution of some lineages of *Rhinella*; and (4) the incorporation of more phenotypic characters to better understand their evolution in this group and define many morphologically and ecologically diverse clades of the genus. Future studies addressing these problems would result in crucial contributions in the knowledge of the diversity of *Rhinella*.

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APPENDIX 1

Locality Data of Voucher Specimens and Sources of the Sequences

Collection abbreviations are as follow: **AG,** Anna Goebel field series; **AACRG,** African Amphibian Conservation Research Group, North-West University, Potchefstroom, South Africa; **AF,** Antoine Fouquet field series; **AJC,** Andrew J. Crawford field series; **AML,** Alejandro Montoya L. field series; **AMNH,** American Museum of Natural History, New York; **AMNH-FS,** American Museum of Natural History field series, New York; **ANDES,** Museo de Historia Natural Andes, Universidad de los Andes, Bogotá, Colombia; **APL,** Albertina P. Lima field series; **AR,** Alexander Robertson field series; **BB,** Boris Blotto field series; to be accessioned in MACN; **BM,** Michel Blanc field series; **BMNH,** Natural History Museum, London, UK; **CAS,** California Academy of Sciences, San Francisco, California; **CBA,** César Barrio-Amoros field series; **CFBH,** Collection Célio F.B. Haddad, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil; **CFBH-T,** Célio F.B. Haddad tissue collection, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil; **CH,** Círculo Herpetológico de Panamá, Panamá, Panama; **CHUNB,** Coleção Herpetológica da Universidade de Brasília, Brasília, Brazil; **CORBIDI,** Centro de Ornitología y Biodiversidad, Lima, Peru; **CTGA-UFAM,** tissues collection of Universidade Federal do Amazonas, Manaus, Amazonas, Brazil; **CZUT,** Colección Zoológica, Facultad de Ciencias, Universidad del Tolima, Tolima, Colombia; **DCC,** David C. Cannatella field series; **DPL,** Dwight P. Lawson field series; **ESTR,** locality code; (Miguel Trefaut Rodrigues field series) **FML,** Fundación Miguel Lillo, San Miguel de Tucumán, Argentina; **FMNH,** Field Museum, Chicago, IL; **ICN,** Universidad Nacional de Colombia, Instituto de Ciencias Naturales, Museo de Historia Natural, Bogotá, Colombia; **IDLR,** Ignacio de la Riva field series; **IIBP,** Instituto de Investigación Biológica del Paraguay, Asunción; **IWU,** Illinois Wesleyan University, Bloomington, IN; **IZUA,** Instituto de Zoología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile; **JMP,** José M. Padial field series; **KMH,** Kim M. Howell field series; **KRL,** Karen R. Lips field series; **KU,** University of Kansas Natural History Museum, Lawrence, Kansas, KS; **LAJ,** locality code; **LGE,** Laboratorio de Genética Evolutiva, Universidad Nacional de Misiones, Argentina; **LSUMZ,** Louisiana State University, Museum of Natural Science, Baton Rouge, Louisiana, LA; **MACN,** Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"–CONICET, Buenos Aires, Argentina; **MAR,** Marco A. Rada field series; **MC,** Christian Marty field series; **MCZ,** Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, MA; **MHNLS,** Museo de Historia Natural La Salle, Fundación La Salle de Ciencias Naturales, Caracas, Venezuela; **MHUA,** Museo de Herpetología de la Universidad de Antioquia, Medellín, Colombia; **MJH,** Martin J. Henzl field series; **MNCN,** Museo Nacional de Ciencias Naturales, Madrid, Spain; **MNCN-ADN,** Museo Nacional de Ciencias Naturales tissue collection, Madrid, Spain; **MNHN-Fr,** Muséum national d'Histoire naturelle, Paris, France; **MNHN-Uy,** Museo Nacional de Historia Natural, Montevideo, Uruguay; **MNRJ,** Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil; **MTD,** Senckenberg Naturhistorische Sammlungen Dresden, Dresden, Germany; **MTR,** Miguel Trefaut Rodrigues field series; **MTSN,** Museo Tridentino di Scienze Naturali, Trento, Italy; **MUBI,** Museo de Biodiversidad del Perú, Cusco, Peru; **MUSM,** Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; **MVUP,** Museo de Vertebrados, Universidad de Panamá, Panamá, Panama; **MVZ,** University of California, Museum of Vertebrate Zoology, Berkeley, CA; **MW,** Mark Wilkinson field series; **MZUSP,** Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; **NB,** Néstor Basso field series; **MNK,** Museo de Historia Natural "Noel Kempff Mercado", Santa Cruz de la Sierra, Bolivia; **NMP,** Národní muzeum National Museum, Prague, Czech Republic; **NP,** Nikolai Poyarkov field series;

PD, Pedro Dias field series; **PG,** Philippe Gaucher field series; **QCAZ,** Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Pichincha, Ecuador; **RGP,** Roberto Gutierrez Poblete field series; **ROM,** Royal Ontario Museum, Department of Natural History, Toronto, Canada; **SBH,** S. Blair Hedges field series; **SMF,** Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt am Main, Germany; **TG,** Taran Grant field series; **TWR,** Tod W. Reeder field series; **UFMT,** Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil; **UFRGS,** Universidade Federal do Rio Grande do Sul, Departamento de Zoologia, Rio Grande do Sul, Brazil; **UNSJ,** Universidad Nacional de San Juan, San Juan, Argentina; **URCA,** Universidade Regional do Cariri, Crato, Ceará, Brazil; **USNM,** National Museum of Natural History, Smithsonian Institution, Washington DC; **UTA,** University of Texas at Arlington Department of Biology, Arlington, TX; **UWIZM,** University of the West Indies, Zoology Museum, Department of Life Sciences, St. Augustine, Saint George, Trinidad and Tobago; **VG,** Václav Gvoždík field series; **VUB,** Vrije Universiteit Brussel, Belgium; **ZUEC,** Museu de História Natural, Universidade Estadual de Campinas, Campinas, Brazil; **ZUFG,** Universidade Federal de Goiás, Goiânia, state of Goiás, Brazil; and **ZVC,** Colección de Zoología Vertebrados de la Facultad de Ciencias, Montevideo, Uruguay. Abbreviations: nd, no data; nv, no voucher specimen; —, no change in the taxonomy of the species.

RHINELLA

Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. abei	*R. ornata*	CFBH 18141	Brazil: Paraná: Quatro Barras	This study; Thomé et al., 2010
		MACN 46672	Brazil: Santa Catarina: Garopaba	This study
		MZUSP 128425	Brazil: Paraná: Wenceslau Brás	This study; Thomé et al., 2010
R. achalensis	—	MACN 52406	Argentina: San Luis: La Carolina	This study
R. achavali	—	MNHN-Uy 9301	Uruguay: Treinta y Tres: Quebrada de los Cuervos	This study
		ZVC 3801	Uruguay: Treinta y Tres: Estancia Doña Alba	Vallinoto et al., 2010
R. acrolopha	—	MAR 1425	Colombia: Chocó: Unguia	This study
		MAR 1426	Colombia: Chocó: Unguia	This study
R. acutirostris	"*R. acutirostris*"	CORBIDI 4635	Peru: Loreto: Andoas	This study
		MTR 36593	Brazil: Amazonas: Rio Içá	This study
		MTR 36684	Brazil: Amazonas: Rio Içá	This study
		QCAZ 10601	Ecuador: Orellana: Parque Nacional Yasuní, Estación Científica Yasuní PUCE	Pramuk, 2006; Pramuk et al., 2008

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RHINELLA Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. acutirostris	"*R. acutirostris*"	QCAZ 28379	Ecuador: Sucumbios: Reserva de Producción Faunística Cuyabeno, Playas de Cuyabeno	This study
R. alata	"*R. alata*"	CH 9192	Panama: Colón: Parque Nacional Soberania	Santos et al., 2015
		MAR 2574	Colombia: Tolima: Rioblanco	This study
		MHUA 8415	Colombia: Antioquia: Sonsón	This study
		QCAZ 11597	Ecuador: Esmeraldas: Bosque Protector La Chiquita	Pramuk, 2006; Pramuk et al., 2008
		QCAZ 13896	Ecuador: Cañar: Manta Real	Pramuk, 2006; Pramuk et al., 2008
R. amabilis	*R.* cf. *amabilis*	QCAZ 68471	Peru: Cajamarca	This study
R. amboroensis	*R. quechua*	MNK 5302	Bolivia: Santa Cruz: Parque Nacional Amboró	Frost et al., 2006
R. arborescandens	—	CORBIDI 2020	Peru: Amazonas: Bagua, Cataratas de Camñopite	This study
		MUBI 14076	Peru: Amazonas: Bongara	This study
		MUBI 14082	Peru: Amazonas: Bongara	This study
R. arenarum	—	AR 305	Argentina	Pramuk, 2006; Pramuk et al., 2008
R. arenarum arenarum	*R. arenarum*	MNHN-Uy 9935	Uruguay: Cerro Largo: Laguna Merín	This study
		MACN 38639	Argentina: San Luis: Lomas Blancas	This study; Faivovich et al., 2005; Frost et al., 2006
		MNCN-ADN 5972	Bolivia: Tarija: Reserva Tariquia	This study
R. arenarum mendocina	*R. arenarum*	MACN 49141	Argentina: Mendoza: Tunuyán	This study
R. arequipensis	*R. spinulosa*	KU 214792	Peru: Arequipa: Zamacola, Cerro Colorado	Pramuk, 2006; Pramuk et al., 2008
		LGE 2516	Peru: Arequipa: Cañón del Colca	This study
R. arunco	—	KU 217369	Chile: Santiago: Rungue	Pramuk, 2006; Pramuk et al., 2008

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RHINELLA

Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. atacamensis	—	AMNH 168401	n/d	Frost et al., 2006
		KU 217352	Chile: Coquimbo: Cuesta Pajonales	Pramuk, 2006; Pramuk et al., 2008
R. azarai	—	LGE 8710	Argentina: Misiones: Candelaria	Pereyra et al., 2016a
		LGE 8711	Argentina: Misiones: Candelaria	Pereyra et al., 2016a
R. beebei	—	CBA 5732	Venezuela: Bolivar: Chivatón, Gran Sabana	Pereyra et al., 2016a
		ICN 55776	Colombia: Casanare: Paz de Ariporo, Vereda La Colombina, Finca El Porvenir	Murphy et al., 2017
		ICN 55784	Colombia: Casanare: Trinidad, Vereda La Cañada, Finca La Palmita	Murphy et al., 2017
		nv	Venezuela: Amazonas: Puerto Ayacucho	Pereyra et al., 2016a
		UWIZM 2012.27.72.3	Trinidad and Tobago: Trinidad: Trincity Central Road	Murphy et al., 2017
R. bergi	—	LGE 8723	Argentina: Formosa: Pilcomayo, Palma Sola	Pereyra et al., 2016a
		MACN 46555	Argentina: Chaco: San Fernando	Pereyra et al., 2016a
R. bernardoi	—	FML 23921	Argentina: San Juan: Parque Provincial Ischigualasto	Pereyra et al., 2016a
		UNSJ 5046	Argentina: San Juan: Caucete	Pereyra et al., 2016a
R. casconi	—	CFBH 22863	Brazil: Ceará: Guaramiranga	This study
		CFBH 22865	Brazil: Ceará: Guaramiranga	This study
R. castaneotica	—	LSUMZ 17429	Brazil: Pará: 100 km S Santarém	Pramuk, 2006; Pramuk et al., 2008
R. cf. *castaneotica*	"*R. castaneotica*"	NMP6V 74261	Bolivia: Pando, Federico Román: Santa Crucito	Moravec et al., 2014
		BM 131	French Guiana: Mataroni	Fouquet et al., 2007c
		ZUFG 8171	Brazil: Acre: Boca do Acre	This study

APPENDIX 1 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. cf. *castaneotica*	*R. proboscidea*	MTR 10003	Brazil: Amazonas: Lago Cipotuba	Fouquet et al., 2012a
R. centralis	—	CH 9383	Panama: Coclé: Valle de Antón	Pereyra et al., 2016a
		MVUP 2305	Panama: Coclé: Valle de Antón	Pereyra et al., 2016a
R. ceratophrys	*Rhaebo ceratophrys*	JMP 2284	Colombia: Amazonas: Leticia	This study
		QCAZ 40240	Ecuador: Sucumbios: Sansa Huari, Comuna Singue 1	This study
R. cerradensis	—	CFBH 20517	Brazil: Bahia: Jaborandi	This study
		CHUNB 38671	Brazil: Distrito Federal: Brasília	This study
		CHUNB 39953	Brazil: Distrito Federal: Brasília	This study
R. aff. *cerradensis*	—	LGE 19096	Argentina: Misiones: Posadas	This study
		LGE 19103	Argentina: Misiones: Posadas	This study
		MNHN-Uy 9514	Uruguay: Rivera: Pueblo Madera	This study
R. chavin	—	MTD 43789	Peru: Huánuco: Pachitea, Palma Pampa	Pramuk, 2006; Pramuk et al., 2008
R. crucifer	—	CFBH 2867	Brazil: Espírito Santo: Aracruz	This study; Thomé et al., 2010
		CFBH 24630	Brazil: Bahia: Camacan	This study; Thomé et al., 2010
R. dapsilis	—	QCAZ 17719	Ecuador: Napo: Cando	Pramuk, 2006; Pramuk et al., 2008
		QCAZ 38892	Ecuador: Pastaza: Villano	Santos et al., 2015
		QCAZ 43967	Ecuador: Orellana: Parque Nacional Yasuní, Comunidad Añangu, Río Napo	This study
R. cf. *dapsilis*	*R. dapsilis*	CORBIDI 1969	Peru: Amazonas: Bagua, Chonza Alta	This study
		MTR 6313	Brazil: Pará: Serra do Kukoinhokren	Fouquet et al., 2012a
		MZUSP 139598	Brazil: Pará	This study
		QCAZ 38621	Ecuador: Pastaza, Villano	Santos et al., 2015

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RHINELLA

Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. cf. *dapsilis*	*R. dapsilis*	QCAZ 39474	Ecuador: Orellana: Alta Florencia, 6.5 km NO de Nuevo Rocafuerte, Río Napo	This study
R. diptycha	—	KU 289057	Paraguay: Concepción: Parque Nacional Serranía San Luis	Mulcahy et al., 2006
		MACN 51118	Argentina: Santiago del Estero: Guasayán	This study; Frost et al., 2006
		MNCN-ADN 6044	Bolivia: La Paz: San José de Uchupiamonas	This study; Vallinoto et al., 2010
R. cf. *diptycha*	*R. diptycha*	LGE 9867	Argentina: Misiones: Capital, Fachinal	This study
R. dorbignyi	—	MACN 43695	Argentina: Buenos Aires: Dolores	Pereyra et al., 2016a
		MNHN-Uy 9492	Uruguay: Treinta y Tres: Bañado de los Oliveras	Pereyra et al., 2016a
R. cf. *dorbignyi*	*R. dorbignyi*	CFBH 14062	Brazil: Rio Grande do Sul: Rio Grande	Pereyra et al., 2016a
R. fernandezae	*R. dorbignyi*	LGE 8717	Argentina: Corrientes: General Paz, Itá Ibaté	Pereyra et al., 2016a
		LGE 8718	Argentina: Santa Fe: 9 de Julio, Tostado	Pereyra et al., 2016a
R. festae	—	CORBIDI 7505	Peru: Loreto: Datem del Marañon, Morona	This study
		KU 217501	Ecuador: Pastaza: Montalvo	Pramuk, 2006; Pramuk et al., 2008; Mendelson et al., 2011
		QCAZ 18203	Ecuador: Napo: Estación Biológica Jatun Sacha	Santos et al., 2015
		QCAZ 41490	Ecuador: Zamora: Miazi Alto	This study
		QCAZ 46457	Ecuador: Morona: Santiago, Nuevo Israel	This study
R. fissippes	—	MNCN-ADN 6310	Bolivia: Beni-Cochabamba: Santo Domingo, Parque Nacional Isiboro-Sécure	This study
R. gallardoi	—	LGE 4546	Argentina: Jujuy: Manuel Belgrano	This study
		LGE 4735	Argentina: Jujuy: Abra Colorada	This study

APPENDIX 1 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. gildae	*R. dapsilis*	CFBH 11400	Brazil: Tocantins: Babaçulândia	This study
		ESTR 173	Brazil: Maranhão: Carolina	Fouquet et al., 2012b
		URCA 12651	Brazil: Maranhão: São Pedro da Água Branca	Avila et al., 2018
R. granulosa	—	CFBH 7341	Brazil: Alagoas: Passo de Camaragibe	Pereyra et al., 2016a
		CFBH 18706	Brazil: Espírito Santo: Linhares	Pereyra et al., 2016a
R. henseli	—	CFBH 20117	Brazil: Rio Grande do Sul: Catiporã	This study
		MNRJ 33006	Brazil: Rio Grande do Sul: Mato Castelhano	Thomé et al., 2010; Pereyra et al., 2016a
		UFRGS 3569	Brazil: Rio Grande do Sul: Nova Roma do Sul	This study
R. hoogmoedi	—	CFBH 13286	Brazil: Bahia: Una	This study
		CFBH 15962	Brazil: São Paulo: Santos	This study
		MTR 16199	Brazil: Bahia: Serra Bonita, Camacan	Fouquet et al., 2012b
		ZUECDCC 3393	Brazil: Rio de Janeiro: Magé, Santo Aleixo	Pauly et al., 2004
R. horribilis	—	KRL 744	Panamá: Coclé: El Cope	Crawford et al., 2010
		KU 289750	El Salvador: Ahuachapan: Parque Nacional El Imposible	Mulcahy et al., 2006; Pramuk, 2006; Pramuk et al., 2008
		MAR 2057	Colombia: Valle del Cauca: Dagua	This study
		UTA 54882	Mexico: Veracruz	Mulcahy et al., 2006
	Rhinella sp. 1	KU 202274	Ecuador: Pichincha: Tinalandia	Pauly et al., 2004
		KU 217482	Ecuador: Loja: Vilcabamba	Mulcahy et al., 2006; Pramuk, 2006; Pramuk et al., 2008
		QCAZ 47444	Ecuador: Loja: San Bernabé	This study
		QCAZ 50698	Ecuador: Manabí: Puerto Cayo	This study
R. humboldti	"*R. humboldti*"	AJC 3533	Colombia: Santander: San Vicente de Chucuri, Reserva El Arboretum	Guarnizo et al., 2015

APPENDIX 1 *continued*

RHINELLA

Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. humboldti	*"R. humboldti"*	CZUT 1717	Colombia: Tolima: Prado, Vereda El Caimán, Represa hidroeléctrica Hidroprado	Murphy et al., 2017
R. icterica	*"R. icterica"*	CFBH 11027	Brazil: Santa Catarina: Bom Jardim da Serra, Serra do Rio do Rastro	This study; Thomé et al., 2010
		CFBH 13965	Brazil: Rio de Janeiro: Petrópolis	This study
		CFBH 27410	Brazil: Rio de Janeiro: Parque Estadual dos Três Picos	This study
		CFBH 38392	Brazil: Minas Gerais: Rio Preto	This study
R. cf. *icterica*	*"R. icterica"*	MACN 43789	Argentina: Misiones: San Vicente	This study
R. inca	—	CORBIDI 6920	Peru: Ayacucho: San Antonio, La Mar	This study
		LGE 2554	Peru: Cusco: Urubamba	This study
		MNCN 44405	Peru: Cusco: La Convención, Río Kimbiri, Comunidad Machiguenga Pomoreni	This study
		MNCN 44406	Peru: Cusco: La Convención: Río Kimbiri, Comunidad Machiguenga Pomoreni	This study
R. inopina	—	CHUNB 51110	Brazil: Bahia: São Desidério	This study
		MZUSP 142356	Brazil: Minas Gerais: Januaría	This study
		MZUSP 142094	Brazil: Minas Gerais: Januaría	This study
R. jimi	*R. diptycha*	CFBH 19335	Brazil: Bahia: Maracás	This study
		CFBH 19523	Brazil: Bahia: Maracás	This study
R. justinianoi	—	MNCN-ADN 6065	Bolivia: Santa Cruz: Florida, La Yunga de Mairana	This study
R. leptoscelis	—	MUBI 5976	Peru: Puno: Carabaya	This study
		MUBI 5989	Peru: Puno: Carabaya	This study
	Rhinella sp. 2	CORBIDI 7266	Peru: Oxapampa: Huancabamba, Huampal	This study

APPENDIX 1 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. leptoscelis	*Rhinella* sp. 2	MUBI 14523	Peru: Pasco: Oxapampa	This study
		MUSM 31150	Peru: Pasco: Oxapampa, Oxapampa	Moravec et al., 2014
		NMP6V 74749	Peru: Pasco: Oxapampa, Quebrada San Alberto	Moravec et al., 2014
R. lescurei	—	AF 1613	French Guiana: St Laurent Du Maroni Saul	This study
		MC 5	French Guiana: Cisame	Fouquet et al., 2007c
		MNHN-Fr 2006.2611	French Guiana: Haute Wanapi	Fouquet et al., 2012a
R. lilyrodriguezae	—	CORBIDI 6778	Peru: San Martín: Mariscal Caceres	This study
		CORBIDI 6780	Peru: San Martín: Mariscal Caceres	This study
		CORBIDI 8839	Peru: San Martín: Alto Biavo, Parque Nacional Cordillera Azul	This study
		MUSM 32205	Peru: San Martín: Alto Biavo, Parque Nacional Cordillera Azul	Cusi et al., 2017
R. limensis	—	nv	Peru: Lima: Lima	This study
		KU 215587	Peru: Ancash: Casma, Rio Casma, Casma	Pramuk, 2006; Pramuk et al., 2008
		RGP 4719	Peru: Arequipa: Islay	This study
R. lindae	—	MAR 3330	Colombia Antioquia: Parque Nacional Natural Las Orquídeas	This study
		MAR 3431	Colombia: Antioquia: Parque Nacional Natural Las Orquídeas	This study
		MAR 3432	Colombia: Antioquia: Parque Nacional Natural Las Orquídeas	This study
R. macrorhina	—	MAR 2867	Colombia: Caldas: Parque Nacional Selva de Florencia	This study
		MAR 2903	Colombia: Caldas: Parque Nacional Selva de Florencia	This study

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RHINELLA				
Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. macrorhina	—	MHUA 8319	Colombia: Antioquia: Vereda Santa Rita, Guatapé	This study
		MHUA 10262	Colombia: Antioquia: Vereda La Esperanza, El Carmen de Viboral	This study
R. magnussoni	—	APL 20530	Brazil: Pará: Treviso	This study
R. major	—	LGE 8720	Argentina: Salta: Rivadavia, El Ocultar	Pereyra et al., 2016a
		MNCN-ADN 6232	Bolivia: Cochabamba: Chapare	Pereyra et al., 2016a
R. manu	—	MUBI 11372	Peru: Cusco: Trocha Unión	This study
		MNCN-ADN 20672	Peru: Cusco: Parque Nacional Manu	This study
	Rhinella sp. 3	CORBIDI 5152	Peru: Madre de Dios: Tambopata, Baltimore	This study
		MUBI 10487	Peru: Cusco: La Convencion	This study
R. cf. *margaritifera*	*R. dapsilis*	IWU 334	Peru: Junín: Chanchamayo, Ayte, Bosque de Protección Pui Pui	This study; Cusi et al., 2017
		MUSM 32715	Peru: Cusco: Oxapampa	This study; Cusi et al., 2017
	Rhinella sp. 6	ANDES 1723	Colombia: Amazonas: Leticia	This study
	Rhinella sp. 7	PD 16	Brazil: Amazonas: Rio Içá	This study
	Rhinella sp. 10	QCAZ 42269	Ecuador: Napo: Reserva Ecológica Yachana	This study
	Rhinella sp. 11	CHUNB 32342	Brazil: Amazonas: Humaitá	This study
	Rhinella sp. 12	NMP6V 74260	Bolivia: Pando: Manuripi, San Antonio	Moravec et al., 2014
		ROM 40103	Peru: Madre de Dios: Tambopata	Fouquet et al., 2012b
		USNM 268828	Peru: Madre de Dios: Reserva Tambopata	Pramuk, 2006; Pramuk et al., 2008
	Rhinella sp. 13	CORBIDI 5840	Peru: Loreto: Curupa	Santos et al., 2015
		CORBIDI 5848	Peru: Loreto: Curupa	This study
	Rhinella sp. 14	MUBI 6374	Peru: Loreto: Mayna	This study
		MUBI 14775	Peru: Ucayali	This study

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RHINELLA

Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. cf. *margaritifera*	*Rhinella* sp. 14	MUBI 14776	Peru: Ucayali	This study
		CORBIDI 5468	Peru: Cusco: La Convención	This study
		KU 215145	Peru: Madre de Dios: Cusco Amazónico, Puerto Maldonado	Pramuk, 2006; Pramuk et al., 2008
		KU 215146	Peru: Madre de Dios: Cusco Amazónico, Puerto Maldonado	Mendelson et al., 2011
		MNCN-ADN 20639	Peru: Puno: Carabaya, between Puerto Leguia and San Gabán	This study
		NMP6V 74915	Peru: Ucayali: Pucallpa, Masisea	Moravec et al., 2014
R. marina	—	MAR 1982	Colombia: Caquetá: Florencia	This study
		SBH 190696	Jamaica: St. Mary: Galina	Pramuk et al., 2001
		VUB 1965	Suriname	van Bocxlaer et al., 2009; Liedtke et al., 2016
R. martyi	*R. margaritifera*	MC 156	French Guiana: Trijonction	Fouquet et al., 2007c
		MNHN-Fr 2006.2602	Suriname: Brownsberg Nature Park	Fouquet et al., 2012a
		MW 1006	Guyana	van Bocxlaer et al., 2009; van Bocxlaer et al., 2010
R. merianae	—	CFBH 16641	Brazil: Amazonas: Manaus, Reserva Ducke	Pereyra et al., 2016a
		MTR 20517	Brazil: Roraima: Estação Ecológica de Maracá	This study
R. mirandaribeiroi	—	CFBH 10254	Brazil: Tocantins: Araguacema	Pereyra et al., 2016a
		CFBH 13849	Brazil: Marãnhao: Parque Nacional dos Lençóis Maranhenses	Pereyra et al., 2016a
R. multiverrucosa	*R.* cf. *multiverrucosa*	MUBI 11455	Peru: Huanúnco	This study
R. nesiotes	—	CORBIDI 8122	Peru: Cusco: La Convención, Echarati	This study
		CORBIDI 13953	Peru: Huanúco: Puerto Inca, Yuyapichis	This study
R. nicefori	*R.* cf. *nicefori*	MHUA 4793	Colombia: Antioquia: Belmira	This study

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RHINELLA Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. ocellata	—	CFBH 26592	Brazil: Maranhão: Barreirinhas	This study
		LAJ 210	Brazil: Tocantins: Parque Estadual do Lajeado	Fouquet et al., 2012b
		MZUSP 103261	Brazil: Tocantins: Peixe	Pramuk, 2006; Pramuk et al., 2008
		ZUFG 8519	Brazil: Goiás: Morrinhos	This study
R. ornata	—	CFBH 18815	Brazil: Rio de Janeiro: Parque Nacional da Serra dos Órgãos	This study; Thomé et al., 2010
		CFBH 38375	Brazil: Rio de Janeiro: Visconde de Mauá	This study
		LGE 6503	Argentina: Misiones: Cuña Pirú	This study
		LGE 8729	Argentina: Misiones: Profundidad	This study
		LGE 19020	Argentina: Misiones: El Soberbio	This study
		LGE 19027	Argentina: Misiones: Capital	This study
		USNM 303015	Brazil: São Paulo: Salesópolis, Serra do Mar	Mulcahy et al., 2006; Pramuk, 2006
		ZUECDCC 3392	Brazil: Rio de Janeiro: Magé, Campo de Escoteiros, Santo Aleixo	Pauly et al., 2004; Brandvain et al., 2014
R. paraguas	—	TG 1415	Colombia: Valle del Cauca: El Cairo	This study
		TG 1480	Colombia: Valle del Cauca: El Cairo	This study
R. paraguayensis	*R. scitula*	UFMT 1876	Brazil: Mato Grosso: Poconé	This study
R. cf. *paraguayensis*	*R. stanlaii*	SMF 88237	Bolivia: Santa Cruz: Ñuflo de Chavez, San Sebastián	Jansen et al., 2011
R. poeppigii	—	MUBI 6863	Peru: Cusco: Quispicanchis	This study
		MUBI 6864	Peru: Cusco: Quispicanchis	This study
		USNM 268824	Peru: Madre de Dios: Puerto Maldonado	Pramuk, 2006; Pramuk et al., 2008, Brandvain et al., 2014

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RHINELLA

Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. proboscidea	—	AMNH-FS 20085	Brazil: Roraima: Floresta	This study
		CTGA-UFAM 5602	Brazil: Amazonas: Universidade Federal do Amazonas, near Manaus	Motta et al., 2018
	Rhinella sp. 8	QCAZ 28573	Ecuador: Sucumbios: Sucumbíos: Campo Vinita, vía Palma Roja-Pto El Carmen de Putumayo	This study
	Rhinella sp. 9	CORBIDI 102	Peru: Loreto: Maynas	This study
		CORBIDI 5835	Peru: Loreto: Maynas	This study
		MNCN-ADN 26559	Peru: Loreto: Maronal, Río Ampiyacu	This study
R. pygmaea	—	CFBH 2894	Brazil: Rio de Janeiro: Sao João da Barra	Pereyra et al., 2016a
		CFBH-T 15163	Brazil: Espírito Santo: Mimoso do Sul	This study, Pereyra et al., 2016a
R. quechua	—	MNCN-ADN 3927	Bolivia: Cochabamba: Parque Nacional Carrasco, Sehuencas	This study
R. rubescens	—	CFBH 5836	Brazil: Minas Gerais: Poços de Caldas	This study
		CFBH 7696	Brazil: Goiás: Cocalzinho de Goiás	This study; Thomé et al., 2010
R. rubropunctata	—	MACN 52275	Argentina: Chubut: Cushamen	This study
R. ruizi	—	AML 39	Colombia: Antioquia: Medellín	This study
		AML 40	Colombia: Antioquia: Medellín	This study
R. rumbolli	—	MACN 53782	Argentina: Salta: Santa Victoria, Parque Nacional Baritú	This study
		MACN 43719	Argentina: Salta: Santa Victoria, Parque Nacional Baritú	This study
R. scitula	—	IIBP 849	Paraguay: Concepción	This study
R. cf. *scitula*	*R. scitula*	CFBH 42359	Brazil: Mato Grosso do Sul: Corumbá	This study
R. sclerocephala	—	MHNLS 7495	Venezuela: Cojedes: Cerro Azul, fila La Blanquera	This study

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RHINELLA

Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. spinulosa papillosa	*R. papillosa*	BB 983	Argentina: Neuquén: Minas	This study
		BB 1032	Argentina: Rio Negro: Bariloche, Pampa Linda	Frost et al., 2006
		MACN 49782	Argentina: Chubut: Lago Puelo	This study
		NB 96-23	Argentina: Neuquén: Laguna Blanca	Pauly et al., 2004, Brandvain et al., 2014
R. spinulosa spinulosa	*R. spinulosa*	IDLR 3837	Bolivia: La Paz: stream between Charazani and Curva	Pramuk, 2006; Pramuk et al., 2008
		MUBI 10737	Peru: Cusco: Colcha	This study
		MUBI 10770	Peru: Cusco: Chumbivilcas	This study
		nv	Peru: Puno: Acocollo	This study
	R. altiperuviana	MACN 49701	Argentina: Jujuy: Tumbaya, Quebrada de Sepultura	This study
		MNCN 41989	Bolivia: La Paz: Inquisivi, Quebrada entre Quime e Inquisivi	This study
R. spinulosa trifolium	*R. trifolium*	CORBIDI 5530	Peru: Lima: Huancaya, Vilca	This study
		nv	Peru: Junín: Huancayo	This study
R. stanlaii	—	ZUFG 6456	Brazil: Mato Grosso: Tangará da Serra	This study
		MNCN-ADN 4160	Bolivia: Santa Cruz: Amboró, Ichilo	This study
		MNCN-ADN 6274	Bolivia: Cochabamba: Carrasco, Chaquisacha	This study
R. aff. *stanlaii*	*Rhinella* sp. 15	MNCN-ADN 4159	Bolivia: La Paz: Parque Nacional Madidi, Serranía Sadiri	This study
R. sternosignata	—	nv	Venezuela: Barinas: Cano Los Monos, Acequias	Pereyra et al., 2016a
R. cf. *sternosignata*	*Rhinella* sp. 13	MAR 1314	Colombia: Boyacá: Pajarito	This study
		MAR 1955	Colombia: Caquetá: Florencia	This study

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RHINELLA Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. tacana	—	MUBI 6950	Peru: Cusco: Quispicanchis	This study
		MUBI 7409	Peru: Cusco: Quispicanchis	This study
		MUBI 7007	Peru: Cusco: Quispicanchis, Camanti	This study
		MNK 7187	Bolivia: La Paz: Parque Nacional Madidi	This study
		UTA 53310	Bolivia: La Paz	Pramuk, 2006; Pramuk et al., 2008
R. tenrec	—	MAR 3584	Colombia: Antioquia: Parque Nacional Las Orquídeas	This study
		MAR 3585	Colombia: Antioquia: Parque Nacional Las Orquídeas	This study
R. vellardi	—	CORBIDI 7626	Peru: La Libertad: Pataz, Vijus	This study
		KU 211765	Peru: Cajamarca: Cajabamba	Pramuk, 2006; Pramuk et al., 2008
		MUBI 14281	Peru: La Libertad: Sánchez Carrion	This study
		MUBI 14291	Peru: La Libertad: Sánchez Carrion	This study
R. veraguensis	—	IDLR 3820	Bolivia: La Paz	Pramuk, 2006; Pramuk et al., 2008
		MNCN-ADN 5808	Bolivia: Cochabamba: Parque Nacional Carrasco, Kharahuasi	This study
		MUBI 5946	Peru: Puno: Santo Domingo	This study
		USNM 346048	Peru: Cusco: Paucartambo	Pramuk, 2006; Pramuk et al., 2008
R. veredas	—	CFBH 20516	Brazil: Bahia: São Desidério	This study
		CHUNB 44609	Brazil: Minas Gerais: Buritizeiro	This study; Maciel et al., 2010
R. yanachaga	—	CORBIDI 7269	Peru: Pasco: Oxapampa	This study
		MUBI 7119	Peru: Pasco: Oxapampa	This study
		MUBI 7121	Peru: Pasco: Oxapampa	This study

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RHINELLA				
Current taxonomy	Updated taxonomy	Voucher	Locality	Sources
R. yunga	*R. iserni*	NMP6V 75552	Peru: Junín: Chanchamayo, Bosque de Protección Pui Pui	This study; Cusi et al., 2017
		MUSM 31096	Peru: Cusco: Oxapampa, Quebrada San Alberto, Parque Nacional Yanachaga-Chemillén	This study
		MUSM 31097	Peru: Cusco: Oxapampa, Quebrada San Alberto, Parque Nacional Yanachaga-Chemillén	This study
		MUSM 31950	Peru: Junín: Chanchamayo, Bosque de Protección Pui Pui	This study
R. sp. "gr. *acrolopha*"	*Rhinella* sp. 4	TG 2115	Colombia: Tolima: Murillo, km 22 carretera Murillo-Manizales	Machado et al., 2016
R. sp. gr. *margaritifera*	*R. dapsilis*	MC 204	French Guiana: Saul	Fouquet et al., 2007c
		PG 144	French Guiana: Patawa	Fouquet et al., 2007c
	Rhinella sp. 5	QCAZ 53072	Ecuador: Pastaza: Comunidad Puka Yaku	This study
		QCAZ 53142	Ecuador: Pastaza: Comunidad Campus	This study
	Rhinella sp. 13	MHNLS 21837	Venezuela: Miranda: Río Araira	This study
R. sp. gr. *marina*	"*R. icterica*"	LGE 19195	Argentina: Misiones: Posadas	This study

OUTGROUPS	Voucher	Locality	Sources
Amazophrynella aff. *minuta*	MJH 7095	Peru: Huanuco: Rio Llullapichis, Panguana	Faivovich et al., 2005; Grant et al., 2006
Anaxyrus americanus	CAS 207258	U.S.: Mississippi: Boswell Lake	van Bocxlaer et al., 2009; Liedtke et al., 2016
Anaxyrus boreas	CAS 201586	U.S.: California: Grover Hot Springs Rd	van Bocxlaer et al., 2009; Liedtke et al., 2016
Anaxyrus quercicus	MVZ 223370	U.S.: Florida: Charlotte	Pauly et al., 2004; Brandvain et al., 2014
Anaxyrus woodhousii	KU 224658	U.S.: Kansas: Barber, Sharon	Pramuk, 2006; Pramuk et al., 2008
Ansonia longidigita	VUB 666	Malaysia: Borneo	van Bocxlaer et al 2009; Liedtke et al 2016

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OUTGROUPS	Voucher	Locality	Sources
Bufo bufo	VUB 982	Belgium	van Bocxlaer et al., 2009
	MVZ 230209	Turkey: Bursa: Bursa	Pramuk, 2006; Pramuk et al., 2008
Bufo gargarizans	CAS 228184	China: Yunnan: Fugong-Gongshan rd, N of Fugong	van Bocxlaer et al., 2009; Liedtke et al., 2016
	USNM 292081	China: Sichuan: Shimian	Pramuk, 2006; Pramuk et al., 2008
Bufotes luristanicus	NP 13-1	nd	van Bocxlaer et al., 2010
Duttaphrynus melanostictus	VUB 52	India	Bossuyt and Milinkovitch, 2000; Biju and Bossuyt, 2003; Roelants et al., 2007; van Bocxlaer et al., 2009
Incilius alvarius	USNM 320001	U.S.: Arizona: Continental	Pramuk, 2006; Pramuk et al., 2008
	TWR 628	U.S.: Arizona: Pima, Why	Wiens et al., 2005
Incilius coniferus	KU 217480	Ecuador: Pichincha: Vicente Maldonado	Pramuk, 2006; Pramuk et al., 2008
Incilius nebulifer	UTA 52489	U.S.: Louisiana: Tangipahoa	Mulcahy and Mendelson, 2000; Mendelson et al., 2011
	DCC 3170	U.S.: Texas: Gulf Coast	Santos and Cannatella, 2011
Incilius valliceps	UTA 13097	Mexico: Chiapas	Mulcahy and Mendelson, 2000, Pauly et al., 2004; Brandvain et al., 2014
Ingerophrynus galeatus	FMNH 256443	Lao PDR: Khammouan: Nakai	Pramuk, 2006; Pramuk et al., 2008
Leptophryne borbonica	VUB 673	Malaysia	Roelants et al., 2007; van Bocxlaer et al., 2009; Liedtke et al., 2016
Melanophryniscus stelzneri	VUB 985	nd	Roelants et al., 2007; van Bocxlaer et al., 2009; Liedtke et al., 2016
Mertensophryne loveridgei	KMH 26653	Tanzania	van Bocxlaer et al., 2009
	MCZ 32084	Tanzania	Liedtke et al., 2016
Nannophryne variegata	IZUA 3198	Chile: XII Region: Puerto Edén, Isla Wellington	Pramuk, 2006; Pramuk et al., 2008
Nectophrynoides tornieri	MW 1822	Tanzania	van Bocxlaer et al., 2009
	BMNH 2005.1375	Tanzania	Roelants et al., 2007
Pelophryne misera	VUB 641	Malaysia: Borneo	van Bocxlaer et al., 2009; Liedtke et al., 2016
Peltophryne empusa	nv	Cuba: Isla de la Juventud: Los Indios	Alonso et al., 2012
	SBH 193517	Cuba: Granma: Bartolome Maso	Pramuk et al., 2001; Landestoy et al., 2018
Peltophryne lemur	AG nd	Puerto Rico	Pramuk, 2006; Pramuk et al., 2008
	SBH 190657	Puerto Rico	Pramuk et al., 2001
Phrynoidis juxtaspera	VUB 649	Malaysia: Borneo	van Bocxlaer et al., 2009, Liedtke et al., 2016

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OUTGROUPS	Voucher	Locality	Sources
Rentapia hosii	VUB 661	Malaysia	Roelants et al., 2007; van Bocxlaer et al., 2009; Liedtke et al., 2016
Rhaebo ecuadorensis	QCAZ 13234	Ecuador: Napo: Talag Alto	Pramuk, 2006; Pramuk et al., 2008
Rhaebo guttatus	LSUMZ 17418	Brazil: Rondônia: Parque Estadual Guajara-Mirim	Pramuk, 2006; Pramuk et al., 2008
Rhaebo nasicus	ROM 20650	Guyana: Mazaruni-Potaro: Tukeit	Pramuk, 2006; Pramuk et al., 2008
Schismaderma carens	MW 4279	Tanzania	van Bocxlaer et al., 2009
Sclerophrys brauni	MW 3840	Tanzania	Van Bocxlaer et al., 2009
	BMNH 2002.350	Tanzania	Roelants et al., 2007; Liedtke et al., 2016
Sclerophrys garmani	CAS 214829	Kenya: Coast Province: Watamu	Pramuk, 2006; Pramuk et al., 2008; van Bocxlaer et al., 2009; Brandvain et al., 2014
Sclerophrys mauritanica	NP 22-1	Morocco	van Bocxlaer et al., 2009
	VG 7-025	Morocco	Liedtke et al., 2016
Sclerophrys regularis	KU 290435	Ghana: Eastern Region: Winneba	Pramuk, 2006; Pramuk et al., 2008
Sclerophrys steindachneri	CAS 214839	Kenya: Coast: Kilifi, Kararacha pond	Pramuk, 2006; Pramuk et al., 2008; van Bocxlaer et al., 2009; Liedtke et al., 2016
Vandijkophrynus robinsoni	CAS 193549	South Africa: Northern Cape: Richtersveld National Park	van Bocxlaer et al., 2010
	AACRG 68	nd	Liedtke et al., 2016
Wolterstorffina parvipalmata	DPL 5101	Cameroon	Frost et al., 2006
	MTSN 5895	Cameroon	van Bocxlaer et al., 2009

APPENDIX 2

GenBank Numbers of the Sequences Employed in This Study

List of mitochondrial and nuclear sequences for each terminal used in the different analyses. The sequences produced for this project are in bold. See appendix 1 for institutional codes details, locality data of voucher specimens, and sources of the sequences. Abbreviations: *12S-16S*: 12S ribosomal, tRNA^{val}, and 16S ribosomal sequences; *cxcr4*: the C-X-C motif chemokine receptor 4 gene; *cytb*: cytochrome b gene; *nd1*: NADH dehydrogenase subunit 1 gene (this fragment usually include a section of 16S, tRNA^{ile}, and tRNA^{leu}); nv: No voucher specimen; *pomc*: proopiomelanocortin A; *rag1*-a: recombination-activating gene 1 (isolate a); *rag1*-b: recombination-activating gene 1 (isolate b); *rho:* rhodopsin gene; *slc8a1:* solute carrier family 8 member A1. ^{R} indicates terminals that were employed in restricted mitochondrial (rM) and nuclear (rN) analyses, ^{TE} indicates terminals that were employed in total evidence (maximum parsimony and maximum likelihood) analyses, * indicates terminal where sequences of nuclear and mitochondrial genomes where analyzed independently in the total evidence analyses, due to the occurrence of mitochondrial introgresions. + Sequences of specimens marked with a plus sign (*R. beebei* ICN 55776 and *R. humboldti* CZUT 1717) were accidentally mixed when they were submitted to GenBank (Jowers, personal commun.). # Sequences under these accession numbers are not contiguous along their entire length (see sequence descriptions in GenBank), as they were generated with nonoverlapping primer pairs, and generate large gaps when aligned together with *12S-16S* complete sequences (see alignments at https://doi.org/10.5531/sd.sp.46).

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. abei	*R. ornata*	CFBH 18141$^{R/TE}$	**MW002838**	**MW002969**	**MW003032**	**MW003105**	GU907381	**MW003274**	**MW003371**	**MW003435**	GU907230
		MACN 46672TE	—	—	**MW003033**	**MW003106**	**MW003196**	—	—	**MW003436**	**MW003613**
		MZUSP 128425$^{R/TE}$	**MW002839**	**MW002970**	**MW003034**	**MW003107**	GU907405	**MW003275**	**MW003372**	**MW003437**	GU907245
R. achalensis	—	MACN 52406$^{R/TE}$	**MW002840**	**MW002971**	**MW003035**	**MW003108**	**MW003197**	**MW003276**	—	**MW003438**	**MW003614**
R. achavali	—	MNHN-Uy 9301$^{R/TE}$	**MW002841**	—	—	—	**MW003198**	—	**MW003373**	**MW003439**	**MW003615**
		ZVC 3801TE	—	—	—	—	—	—	GU178809	GU178787 GU178798	—
R. acrolopha	—	MAR 1425$^{R/TE}$	—	**MW002972**	**MW003036**	—	**MW003199**	**MW003277**	—	**MW003440**	**MW003616**
		MAR 1426TE	—	—	—	—	—	—	—	**MW003441**	—
R. acutirostris	"*R. acutirostris*"	CORBIDI 4635TE	**MW002842**	—	—	—	—	**MW003278**	—	**MW003442**	—
		MTR 36593TE	—	—	—	—	—	—	—	**MW003443**	—

APPENDIX 2 *continued*

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. acutirostris	"*R. acutirostris*"	MTR 36684TE	—	—	—	—	—	—	—	**MW003444**	—
		QCAZ 10601TE	—	DQ158312	—	—	—	—	—	DQ158470	—
		QCAZ 28379$^{R/TE}$	—	**MW002973**	**MW003037**	**MW003109**	**MW003200**	**MW003279**	—	**MW003445#**	—
R. alata	"*R. alata*"	CH 9192TE	—	—	—	—	—	—	—	KR012599 KR012611	—
		MAR 2574TE	—	—	—	**MW003110**	—	**MW003280**	—	**MW003446**	—
		MHUA 8415	**MW002843**	**MW002974**	**MW003038**	—	—	**MW003281**	—	**MW003447#**	**MW003617**
		QCAZ 11597TE	DQ306543	DQ158314	DQ158391	—	—	—	—	DQ158472	—
		QCAZ 13896TE	DQ306554	DQ158313	DQ158390	—	—	—	—	DQ158471	—
R. amabilis	*R.* cf. *amabilis*	QCAZ 68471$^{R/TE}$	**MW002844**	**MW002975**		**MW003111**	—	**MW003282**	—	**MW003448#**	—
R. amboroensis	*R. quechua*	MNK 5302TE	—	—	—	—	DQ284003	—	—	DQ283386	—
R. arborescandens	—	CORBIDI 2020TE	**MW002845**	—	—	—	—	**MW003283**	—	**MW003449#**	—
		MUBI 14076$^{R/TE}$	**MW002846**	**MW002976**	**MW003039**	**MW003112**	**MW003201**	**MW003284**	—	**MW003450**	—
		MUBI 14082TE	—	—	—	—	—	—	—	**MW003451**	—
R. arenarum	—	AR 305TE	DQ306529	DQ158271	DQ158354	—	—	—	—	DQ158429	—
R. arenarum arenarum	*R. arenarum*	MNHN-Uy 9935TE	—	—	—	**MW003113**	—	—	**MW003374**	**MW003452**	—
		MACN 38639TE	**MW002847**	—	—	AY844370	AY844547	—	AY843795	AY843573	JX204061
		MNCN-ADN 5972TE	**MW002848**	—	—	—	—	—	—	**MW003453**	**MW003618**
R. arenarum mendocina	*R. arenarum*	MACN 49141TE	**MW002849**	—	—	**MW003114**	—	—	**MW003375**	**MW003454**	**MW003619**

APPENDIX 2 *continued*

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. arequipensis	*R. spinulosa*	KU 214792$^{\mathrm{TE}}$	DQ306564	DQ158272	DQ158355	—	—	—	—	DQ158430	—
		LGE 2516$^{\mathrm{TE}}$	—	—	—	**MW003115**	**MW003202**	—	—	**MW003455#**	—
R. arunco	—	KU 217369$^{\mathrm{TE}}$	DQ306552	DQ158283	DQ158365	—	—	—	—	DQ158442	—
R. atacamensis	—	AMNH 168401$^{\mathrm{TE}}$	—	—	—	—	DQ283857	—	—	DQ283162	—
		KU 217352$^{\mathrm{TE}}$	DQ306541	DQ158275	DQ158357	—	—	—	—	DQ158433	—
R. azarai	—	LGE 8710$^{\mathrm{R/TE}}$	KP684944	KP685079	KP685115	KP685145	—	—	KP684986	KP685185	KP685036
		LGE 8711$^{\mathrm{R/TE}}$	KP684945	KP685080	KP685116	—	KP685164	KP685017	KP684987	KP685186	KP685037
R. beebei	—	CBA 5732$^{\mathrm{R/TE}}$	KP684964	KP685099	KP685131	KP685153	KP685173	KP685025	—	KP685210	KP685058
		ICN 55776$^{\mathrm{TE}}$ +	—	—	—	—	—	—	—	KU174493 KU174499	—
		ICN 55784$^{\mathrm{TE}}$ +	—	—	—	—	—	—	—	KU174494 KU174500	—
		nv	KP684965	—	—	—	KP685174	—	—	KP685211	—
		UWIZM 2012.27. 72.3$^{\mathrm{TE}}$	—	—	—	—	—	—	—	KU174491 KU174497	—
R. bergi	—	LGE 8723$^{\mathrm{R/TE}}$	KP684946	KP685081	KP685117	KP685146	KP685165	KP685018	KP684988	KP685187	KP685038
		MACN 46555$^{\mathrm{TE}}$	KP684950	KP685084	KP685119	—	—	—	KP684990	KP685192	KP685043
R. bernardoi	—	FML 23921$^{\mathrm{TE}}$ *	KP684952	KP685086	KP685121	—	—	—	KP685193	KP685193	
		UNSJ 5046$^{\mathrm{R/TE}}$ *	KP684951	KP685085	KP685120	KP685148	KP685166	KP685019	—	KP685193	KP685193
R. casconi	—	CFBH 22863$^{\mathrm{R/TE}}$	**MW002850**	**MW002977**	**MW003040**	**MW003116**	**MW003203**	**MW003285**	**MW003376**	**MW003456**	**MW003620**
		CFBH 22865$^{\mathrm{R/TE}}$	**MW002851**	**MW002978**	**MW003041**	**MW003117**	**MW003204**	**MW003286**	—	**MW003457**	**MW003621**

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. castaneotica	—	LSUMZ 17429$^{\mathrm{TE}}$	DQ306539	DQ158282	DQ158364	—	—	—	—	DQ158440	—
R. cf. *castaneotica*	"*R. castaneotica*"	NMP6V 74261$^{\mathrm{TE}}$	—	—	—	—	—	—	—	KF992144	—
		BM 131$^{\mathrm{TE}}$	—	—	—	—	—	—	—	EF364261 EF364287	—
		ZUFG 8171$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003458**	—
	R. proboscidea	MTR 10003$^{\mathrm{TE}}$	—	—	—	—	—	—	—	JN690755 JN691362	—
R. centralis	—	CH 9383$^{\mathrm{TE}}$	KP684954	—	—	—	—	—	—	KP685196	—
		MVUP 2305$^{\mathrm{R/TE}}$	KP684953	KP685087	KP685122	KP685149	KP685167	KP685020	—	KP685195	KP685045
R. ceratophrys	*Rhaebo ceratophrys*	JMP 2284$^{\mathrm{R/TE}}$	**MW002852**	**MW002979**	**MW003042**	**MW003118**	**MW003205**	**MW003287**	**MW003377**	**MW003459**	**MW003622**
		QCAZ 40240$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003460**	—
R. cerradensis	—	CFBH 20517$^{\mathrm{TE}}$	MW002853	—	—	MW003119	MW003206	—	—	**MW003461**	—
		CHUNB 38671$^{\mathrm{TE}}$	MW002854	—	—	MW003120	—	MW003288	—	**MW003462#**	**MW003623**
		CHUNB 39953$^{\mathrm{TE}}$	MW002855	—	—	—	MW003207	MW003289	**MW003378**	**MW003463#**	**MW003624**
R. aff. *cerradensis*	—	LGE 19096$^{\mathrm{R/TE}}$	MW002856	MW002980	MW003043	MW003121	MW003208	MW003290	**MW003379**	**MW003464**	**MW003625**
		LGE 19103$^{\mathrm{R/TE}}$	MW002857	MW002981	MW003044	MW003122	MW003209	MW003291	—	**MW003465**	**MW003626**
		MNHN-Uy 9514$^{\mathrm{R/TE}}$	MW002858	MW002982	MW003045	—	—	—	**MW003380**	**MW003466**	—
R. chavin	—	MTD 43789$^{\mathrm{TE}}$	—	—	—	—	—	—	—	DQ158441	—
R. crucifer	—	CFBH 2867$^{\mathrm{TE}}$	**MW002859**	**MW002983**	—	—	GU907347	—	—	**MW003467**	GU907209
		CFBH 24630$^{\mathrm{R/TE}}$	**MW002860**		—	**MW003123**	GU907425	**MW003292**	**MW003381**	**MW003468**	GU907259

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. dapsilis	—	QCAZ 17719$^{\mathrm{TE}}$	DQ306532	DQ158289	DQ158370	—	—	—	—	DQ158448	—
		QCAZ 38892$^{\mathrm{TE}}$	—	—	—	—	—	—	—	KR012608 KR012640	—
		QCAZ 43967$^{\mathrm{R/TE}}$	**MW002861**	**MW002984**	**MW003046**	**MW003124**	**MW003210**	**MW003293**	—	**MW003469#**	—
R. cf. *dapsilis*	*R. dapsilis*	CORBIDI 1969$^{\mathrm{TE}}$	—	—	—	—	—	**MW003294**	—	**MW003470**	—
		MTR 6313$^{\mathrm{TE}}$	—	—	—	—	—	—	—	JN690787 JN691394	—
		MZUSP 139598$^{\mathrm{TE}}$	**MW002862**		—	—	—	—	—	**MW003471**	**MW003627**
		QCAZ 38621$^{\mathrm{TE}}$	—	—	—	—	—	—	—	KR012606 KR012637	—
		QCAZ 39474$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003472**	—
R. diptycha	—	KU 289057$^{\mathrm{R/TE}}$	DQ306528	DQ158322	KJ609673	KJ609673	—	—	DQ415598	DQ158480 DQ415572	—
		MACN 51118$^{\mathrm{TE}}$	**MW002863**	—	—	—	DQ283791	**MW003295**	—	DQ283065	**MW003628**
		MNCN-ADN 6044$^{\mathrm{TE}}$	**MW002864**	—	—	—	—	**MW003296**	GU178801	**MW003473**	**MW003629**
R. cf. *diptycha*	*R. diptycha*	LGE 9867$^{\mathrm{R/TE}}$	**MW002865**	**MW002985**	—	**MW003125**	**MW003211**	—	—	**MW003473**	—
R. dorbignyi	—	MACN 43695$^{\mathrm{TE}}$	KP684955	KP685089	—	—	—	—	KP684993	KP685198	KP685047
		MNHN-Uy 9492$^{\mathrm{R/TE}}$	KP684956	KP685090	KP685123	—	KP685168	KP685021	KP684994	KP685199	KP685048
R. cf. *dorbignyi*	*R. dorbignyi*	CFBH 14062$^{\mathrm{R/TE}}$	KP684985	KP685112	KP685142	KP685162	—	KP685034	KP685014	KP685232	KP685076
R. fernandezae	*R. dorbignyi*	LGE 8717$^{\mathrm{R/TE}}$	KP684957	KP685091	KP685124	KP685150	—	—	KP684996	KP685201	KP685050
		LGE 8718$^{\mathrm{R/TE}}$	KP684958	KP685092	—	—	KP685169	KP685022	KP684997	KP685202	KP685051

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. festae	—	CORBIDI 7505$^{\mathrm{TE}}$	**MW002866**	—	—	—	—	**MW003297**	—	**MW003475#**	—
		KU 217501$^{\mathrm{TE}}$	DQ306521	DQ158265	DQ158349	—	—	—	—	DQ158423	—
		QCAZ 18203$^{\mathrm{TE}}$	—	—	—	—	—	—	—	KR012609 KR012624	—
		QCAZ 41490$^{\mathrm{R/TE}}$	**MW002867**	**MW002986**	—	**MW003126**	**MW003212**	**MW003298**	—	**MW003476#**	—
		QCAZ 46457$^{\mathrm{TE}}$	**MW002868**	—	—	**MW003127**	—	**MW003299**	—	**MW003477#**	—
R. fissippes	—	MNCN-ADN 6310$^{\mathrm{TE}}$	**MW002869**	—	**MW003047**			**MW003300**	—	**MW003478**	—
R. gallardoi	—	LGE 4546$^{\mathrm{R/TE}}$	**MW002870**	—	**MW003048**	**MW003128**	**MW003213**	**MW003301**	**MW003382**	**MW003479**	**MW003630**
		LGE 4735$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003480#**	—
R. gildae	*R. dapsilis*	CFBH 11400$^{\mathrm{TE}}$	**MW002871**	**MW002987**	—	—	—	—	—	**MW003481**	**MW003631**
		ESTR 173$^{\mathrm{TE}}$	—	—	JN867521	—	—	—	—	JN867548 JN867574	—
		URCA 12651$^{\mathrm{TE}}$	—	—	—	—	—	—	—	MH538281	—
R. granulosa	—	CFBH 7341$^{\mathrm{R/TE}}$	KP684961	KP685095	KP685127	KP685151	KP685170	KP685023	KP685000	KP685205	KP685054
		CFBH 18706$^{\mathrm{R/TE}}$	KP684962	KP685096	KP685128	KP685152	KP685171	KP685024	KP685001	KP685206	KP685055
R. henseli	—	CFBH 20117$^{\mathrm{R/TE}}$	**MW002872**	**MW002988**	**MW003049**	**MW003129**	**MW003214**	**MW003302**	**MW003383**	**MW003482**	**MW003632**
		MNRJ 33006$^{\mathrm{R/TE}}$	KP684942	KP685077	KP685113	KP685143	GU907407	—	—	KP685183	GU907246
		UFRGS 3569$^{\mathrm{R/TE}}$	**MW002873**	**MW002989**	**MW003050**	**MW003130**	**MW003215**	**MW003303**	—	**MW003483**	**MW003633**

APPENDIX 2 *continued*

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. hoogmoedi	—	CFBH 13286$^{R/TE}$	**MW002874**	—	**MW003051**	**MW003131**	**MW003216**	—	**MW003384**	**MW003484**	**MW003634**
		CFBH 15962$^{R/TE}$	**MW002875**	—	**MW003052**	**MW003132**	**MW003217**	**MW003304**	**MW003385**	**MW003485**	**MW003635**
		MTR 16199TE	—	—	JN867518	—	—	—	—	JN867545 JN867571	—
		ZUEC-DCC 3393TE	—	—	—	—	—	—	—	AY680262	—
R. horribilis	—	KRL 744TE *	—	—	—	—	—	—	—	FJ784357	—
		KU 289750TE *	DQ306530	DQ158315	DQ158392	—	—	—	DQ415590	DQ158473	—
		MAR 2057$^{R/TE}$ *	**MW002876**	**MW002990**	**MW003053**	**MW003133**	**MW003218**	**MW003305**	—	**MW003486**	**MW003636**
		UTA 54882TE *	—	—	—	—	—	—	DQ415577	DQ415551	—
	Rhinella sp. 1	KU 202274TE *	—	—	—	—	—	—	—	AY680259	—
		KU 217482TE *	DQ306544	DQ158316	DQ158393	—	—	—	DQ415597	DQ158474	—
		QCAZ 47444$^{R/TE}$ *	**MW002877**	**MW002991**	**MW003054**	**MW003134**	**MW003219**	**MW003306**	—	**MW003487**	—
		QCAZ 50698$^{R/TE}$ *	**MW002878**	**MW002992**	**MW003055**	**MW003135**	—	**MW003307**	—	**MW003488**	—
R. humboldti	"*R. humboldti*"	AJC 3533TE	—	—	—	—	—	—	—	KP149421	—
		CZUT 1717TE +	—	—	—	—	—	—	—	KU174492-KU174498	—
R. icterica	"*R. icterica*"	CFBH 11027$^{R/TE}$	—	**MW002993**	—	**MW003136**	—	**MW003308**	**MW003386**	**MW003489**	GU907200
		CFBH 13965$^{R/TE}$	**MW002879**	—	—	**MW003137**	**MW003220**	**MW003309**	**MW003387**	**MW003490**	**MW003637**
		CFBH 27410$^{R/TE}$	**MW002880**	**MW002994**	**MW003056**	**MW003138**	**MW003221**	**MW003310**	**MW003388**	**MW003491**	**MW003638**
		CFBH 38392TE	—	—	—	—	—	—	—	**MW003492**	**MW003639**

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. cf. *icterica*	"*R. icterica*"	MACN-43789$^{\text{R/TE}}$	**MW002881**	**MW002995**	—	**MW003139**	**MW003222**	**MW003311**	**MW003389**	**MW003493**	**MW003640**
R. inca	—	CORBIDI 6920$^{\text{TE}}$	—	—	—	—	—	—	—	**MW003494#**	—
		LGE 2554$^{\text{TE}}$	**MW002882**	—	—	**MW003140**	—	—	—	**MW003495#**	—
		MNCN 44405$^{\text{R/TE}}$	**MW002883**	**MW002996**	**MW003057**	**MW003141**	**MW003223**	**MW003312**	**MW003390**	**MW003496**	**MW003641**
		MNCN 44406$^{\text{R/TE}}$	**MW002884**	—	—	—	—	—	**MW003391**	**MW003497**	**MW003642**
R. inopina	—	CHUNB 51110$^{\text{R/TE}}$	**MW002885**	**MW002997**	**MW003058**	**MW003142**	—	**MW003313**	—	**MW003498**	**MW003643**
		MZUSP 142356$^{\text{R/TE}}$	**MW002886**	**MW002998**	**MW003059**	**MW003143**	**MW003224**	**MW003314**	—	**MW003499**	**MW003644**
		MZUSP 142094$^{\text{R/TE}}$	**MW002887**	**MW002999**	**MW003060**	**MW003144**	**MW003225**	**MW003315**	—	**MW003500**	**MW003645**
R. jimi	*R. diptycha*	CFBH 19335$^{\text{TE}}$	**MW002888**	—	—	**MW003145**	—	—	—	**MW003501**	—
		CFBH 19523$^{\text{R/TE}}$	**MW002889**	**MW003000**	**MW003061**	**MW003146**	**MW003226**	**MW003316**	**MW003392**	**MW003502**	**MW003646**
R. justinianoi	—	MNCN-ADN 6065$^{\text{R/TE}}$	**MW002890**	**MW003001**	**MW003062**	**MW003147**	**MW003227**	**MW003317**	—	**MW003503**	—
R. leptoscelis	—	MUBI 5976$^{\text{R/TE}}$	**MW002891**	**MW003002**	**MW003063**	**MW003148**	**MW003228**	**MW003318**	**MW003393**	**MW003504**	**MW003647**
		MUBI 5989$^{\text{TE}}$	**MW002892**	—	—	**MW003149**	—	—	**MW003394**	**MW003505#**	**MW003648**
	Rhinella sp. 2	CORBIDI 7266$^{\text{TE}}$	—	—	—	—	—	—	—	**MW003506#**	—
		MUBI 14523$^{\text{R/TE}}$	**MW002893**	**MW003003**	**MW003064**	**MW003150**	**MW003229**	**MW003319**	**MW003395**	**MW003507#**	**MW003649**
		MUSM 31150$^{\text{TE}}$	—	—	—	—	—	—	—	KF992154	—
R. leptoscelis	*Rhinella* sp. 2	NMP6V 74749$^{\text{TE}}$	—	—	—	—	—	—	—	KF992153	—

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. lescurei	—	AF 1613$^{R/TE}$	**MW002894**	**MW003004**	—	**MW003151**	**MW003230**	—	**MW003396**	**MW003508**	**MW003650**
		MC 5TE	—	—	—	—	—	—	—	EF364278 EF364304	—
		MNHN-Fr 2006. 2611TE	—	—	—	—	—	—	—	JN690723 JN691330	—
R. lilyrodriguezae	—	CORBIDI 6778TE	—	—	—	—	—	—	—	**MW003509**#	—
		CORBIDI 6780TE	—	—	—	—	—	—	—	**MW003510**	—
		CORBIDI 8839TE	**MW002895**	—	—	—	—	**MW003320**	—	**MW003511**#	—
		MUSM 32205TE	—	—	—	—	—	—	—	KY912598	—
R. limensis	—	nv$^{R/TE}$	**MW002896**	**MW003005**	**MW003065**	**MW003152**	**MW003231**	**MW003321**	—	**MW003512**	**MW003651**
		KU 215587TE	DQ306509	DQ158307	—	—	—	—	—	DQ158466	—
		RGP 4719TE	**MW002897**	—	—	—	—	—	—	**MW003513**	**MW003652**
R. lindae	—	MAR 3330TE	**MW002898**	—	—	**MW003153**	—	**MW003322**	—	**MW003514**	**MW003653**
		MAR 3431TE	**MW002899**	—	—	**MW003154**	—	—	—	**MW003515**	—
		MAR 3432$^{R/TE}$	**MW002900**	—	**MW003066**	**MW003155**	**MW003232**	—	—	**MW003516**#	—
R. macrorhina	—	MAR 2867TE	—	—	**MW003067**	—	**MW003233**	—	**MW003397**	**MW003517**#	—
		MAR 2903TE	**MW002901**	—	—	—	—	—	—	**MW003518**#	—
		MHUA 8319$^{R/TE}$	—	—	—	—	—	—	—	**MW003519**	—
		MHUA 10262TE	—	—	—	—	—	—	—	**MW003520**	—
R. magnussoni	—	APL 20530TE	**MW002902**	—	—	—	**MW003234**	—	**MW003398**	**MW003521**	—

APPENDIX 2 *continued*

RHINELLA			Nuclear genes						Mitochondrial genes		
Current taxonomy	Updated taxonomy	Voucher	*cxcr4*	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. major	—	LGE 8720$^{\mathrm{TE}}$	KP684966	KP685100	KP685132	—	KP685175	KP685026	KP685003	KP685212	KP685059
		MNCN-ADN 6232$^{\mathrm{TE}}$	KP684973	KP685105	—	—	—	KP685029	KP685008	KP685219	—
R. manu	—	MUBI 11372$^{\mathrm{TE}}$	**MW002903**	**MW003006**	**MW003068**	—	—	—	—	**MW003522**	**MW003654**
		MNCN-ADN 20672$^{\mathrm{R/TE}}$	**MW002904**	**MW003007**	**MW003069**	**MW003156**	**MW003235**	—	**MW003399**	**MW003523**	**MW003655**
	Rhinella sp. 3	CORBIDI 5152$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003524#**	—
		MUBI 10487$^{\mathrm{TE}}$	**MW002905**	—	—	**MW003157**	—	**MW003323**	—	**MW003525**	—
R. cf. *margaritifera*	*R. dapsilis*	IWU 334$^{\mathrm{TE}}$	**MW002906**	—	—	—	—	—	—	**MW003526**	—
		MUSM 32715$^{\mathrm{TE}}$	**MW002907**	—	—	**MW003158**	—	—	**MW003400**	**MW003527**	—
	Rhinella sp. 6	ANDES 1723$^{\mathrm{R/TE}}$	**MW002908**	—	**MW003070**	—	**MW003236**	**MW003324**	**MW003401**	**MW003528**	**MW003656**
	Rhinella sp. 7	PD 16$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003529**	—
	Rhinella sp. 10	QCAZ 42269$^{\mathrm{TE}}$	**MW002909**	—	**MW003071**	—	**MW003237**	**MW003325**	—	**MW003530#**	—
	Rhinella sp. 11	CHUNB 32342$^{\mathrm{R/TE}}$	**MW002910**	—	**MW003072**	**MW003159**	**MW003238**	**MW003326**	**MW003402**	**MW003531**	**MW003657**
	Rhinella sp. 12	NMP6V 74260$^{\mathrm{TE}}$	—	—	—	—	—	—	—	KF992145	—
		ROM 40103$^{\mathrm{TE}}$	—	—	JN867520	—	—	—	JX298409	JN867547 JN867573	—
		USNM 268828$^{\mathrm{TE}}$	DQ306518	DQ158331	DQ158407	—	—	—	—	DQ158490	—

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. cf. *margaritifera*	*Rhinella* sp. 13	CORBIDI 5840TE	—	—	—	—	—	—	—	KR012594 KR012612	—
		CORBIDI 5848TE	**MW002911**	—	—	**MW003160**	—	**MW003327**	—	**MW003532#**	—
		MUBI 6374TE	**MW002912**	**MW003008**	—	—	—	—	—	**MW003533**	**MW003658**
	Rhinella sp. 14	MUBI 14775TE	**MW002913**	**MW003009**	—	—	—	**MW003328**	**MW003403**	**MW003534**	—
		MUBI 14776TE	**MW002914**	—	—	—	—	—	—	**MW003535**	—
		CORBIDI 5468TE	**MW002915**	—	—	**MW003161**	—	**MW003329**	—	**MW003536#**	—
		KU 215145TE	DQ306511	DQ158332	—	—	—	—	—	DQ158491	—
		KU 215146TE	HM563889	—	HM563975	—	—	—	HM563931	HM563816 HM563858	—
		MNCN-ADN 20639$^{R/TE}$	**MW002916**	**MW003010**	**MW003073**	—	**MW003239**	**MW003330**	**MW003404**	**MW003537**	**MW003659**
		NMP6V 74915TE	—	—	—	—	—	—	—	KF992143	—
R. marina	—	MAR 1982$^{R/TE}$	**MW002917**	—	—	**MW003162**	**MW003240**	**MW003331**	—	**MW003538**	—
		SBH 190696TE	—	—	—	—	—	—	AY028510	AY028485 AY028498	—
		VUB 1965TE	KF665869	—	KF666345	—	—	FJ882674	—	FJ882831	FJ882831
R. martyi	*R. margaritifera*	MC 156TE	—	—	—	—	—	—	—	EF364277 EF364303	—
		MNHN-Fr 2006. 2602TE	—	—	—	—	—	—	—	JN690762 JN691369	—
		MW 1006TE	FJ882729	—	—	—	—	FJ882675	—	FJ882832	FJ882832

APPENDIX 2 *continued*

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. merianae	—	CFBH 16641$^{R/TE}$	KP684974	KP685106	KP685137	KP685157	KP685176	KP685030	KP685009	KP685220	KP685065
		MTR 20517$^{R/TE}$	**MW002918**	**MW003011**	—	**MW003163**	—	**MW003332**	**MW003405**	**MW003539**	**MW003660**
R. mirandaribeiroi	—	CFBH 10254$^{R/TE}$	KP684976	KP685108	KP685138	KP685158	KP685177	KP685032	KP685011	KP685223	KP685067
		CFBH 13849$^{R/TE}$	KP684977	KP685109	KP685139	KP685159	KP685178	—	KP685012	KP685224	KP685068
R. multiverrucosa	*R.* cf. *multiverrucosa*	MUBI 11455$^{R/TE}$	**MW002919**	**MW003012**	—	**MW003164**	**MW003241**	**MW003333**	—	**MW003540**	**MW003661**
R. nesiotes	—	CORBIDI 8122TE	—	—	—	—	—	**MW003334**	—	**MW003541#**	—
		CORBIDI 13953TE	—	—	—	—	—	—	—	**MW003542**	—
R. nicefori	*R.* cf. *nicefori*	MHUA 4793TE	—	—	**MW003074**	—	**MW003242**	**MW003335**	—	**MW003543**	—
R. ocellata	—	CFBH 26592$^{R/TE}$	**MW002920**	**MW003013**	**MW003075**	**MW003165**	**MW003243**	**MW003336**	**MW003406**	**MW003544**	**MW003662**
		LAJ 210TE	—	—	JN867519	—	—	—	—	JN867546 JN867572	—
		MZUSP 103261TE	—	DQ158321	DQ158398	—	—	—	—	DQ158479	—
		ZUFG 8519TE	**MW002921**	—	—	—	—	—	—	**MW003545**	**MW003663**
R. ornata	—	CFBH 18815$^{R/TE}$	**MW002922**	**MW003014**	**MW003076**	**MW003166**	—	**MW003337**	**MW003407**	**MW003546**	GU907256
		CFBH 38375TE	—	—	—	—	—	—	—	**MW003547#**	**MW003664**
		LGE 6503TE	**MW002923**	—	—	—	—	—	—	**MW003548**	—
		LGE 8729$^{R/TE}$	**MW002924**	**MW003015**	**MW003077**	—	—	KP685015	—	**MW003549**	**MW003665**
		LGE 19020TE	—	—	—	—	—	—	—	**MW003550**	—

APPENDIX 2 *continued*

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. ornata	—	LGE 19027$^{R/TE}$	**MW002925**	**MW003016**	**MW003078**	**MW003167**	**MW003244**	—	**MW003408**	**MW003551**	**MW003666**
		USNM 303015TE	—	DQ158288	—	—	—	—	DQ415596	DQ158447	—
		ZUECDCC 3392$^{R/TE}$	KJ532322	KJ532293	KJ609675	KJ609675	—	—	—	AY680260	—
R. paraguas	—	TG 1415TE	**MW002926**	—	—	—	—	—	**MW003409**	**MW003552#**	**MW003667**
		TG 1480$^{R/TE}$	—	**MW003017**	—	**MW003168**	**MW003245**	**MW003338**	**MW003410**	**MW003553**	**MW003668**
R. paraguayensis	*R. scitula*	UFMT 1876$^{R/TE}$	**MW002927**	—	—	**MW003169**	**MW003246**	**MW003339**	—	**MW003554**	—
R. cf. *paraguayensis*	*R. stanlaii*	SMF 88237TE	—	—	—	—	—	—	—	JF790186	—
R. poeppigii	—	MUBI 6863$^{R/TE}$	**MW002928**	—	**MW003079**	**MW003170**	**MW003247**	**MW003340**	**MW003411**	**MW003555**	**MW003669**
		MUBI 6864$^{R/TE}$	**MW002929**	—	**MW003080**	**MW003171**	**MW003248**	**MW003341**	—	**MW003556**	**MW003670**
		USNM 268824$^{R/TE}$	DQ306517	KJ532292	KJ609674	KJ609674	—	—	—	DQ158481	—
R. proboscidea	—	AMNH-FS 20085$^{R/TE}$	**MW002930**	**MW003018**	**MW003081**	**MW003172**	**MW003249**	**MW003342**	**MW003412**	**MW003557**	**MW003671**
		CTGA-UFAM 5602$^{R/TE}$	—	—	—	—	—	—	—	MG806046	—
	Rhinella sp. 8	QCAZ 28573$^{R/TE}$	**MW002931**	—	**MW003082**	**MW003173**	**MW003250**	**MW003343**	—	**MW003558#**	—
	Rhinella sp. 9	CORBIDI 102TE	**MW002932**	—	—	—	—	**MW003344**	—	**MW003559#**	—
		CORBIDI 5835TE	—	—	—	—	—	—	—	**MW003560#**	—
		MNCN-ADN 26559$^{R/TE}$	**MW002933**	**MW003019**	—	**MW003174**	**MW003251**	**MW003345**	—	**MW003561**	**MW003672**

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. pygmaea	—	CFBH 2894$^{R/TE}$	KP684982	KP685110	KP685141	KP685160	KP685180	KP685033	KP685013	KP685229	KP685073
		CFBH-T 15163$^{R/TE}$	KP684983	KP685111	—	—	KP685181	**MW003346**	—	KP685230 **MW003562**	KP685074
R. quechua	—	MNCN-ADN 3927$^{R/TE}$	**MW002934**	**MW003020**	**MW003083**	**MW003175**	**MW003252**	**MW003347**	**MW003413**	**MW003563**	**MW003673**
R. rubescens	—	CFBH 5836TE	—	—	—	**MW003176**	**MW003253**	**MW003348**	**MW003414**	**MW003564**	**MW003674**
		CFBH 7696$^{R/TE}$	**MW002935**	—	**MW003084**	**MW003177**		**MW003349**	**MW003415**	**MW003565**	GU907196
R. rubropunctata	—	MACN 52275$^{R/TE}$	**MW002936**	**MW003021**	**MW003085**	**MW003178**	**MW003254**	**MW003350**	**MW003416**	**MW003566**	**MW003675**
R. ruizi	—	AML 39TE	**MW002937**	—	—	—	—	—	—	**MW003567**	—
		AML 40TE	**MW002938**	—	—	—	**MW003255**	**MW003351**	—	**MW003568**	—
R. rumbolli	—	MACN 53782TE	**MW002939**	—	**MW003086**	—	—	—	**MW003417**	**MW003569**	**MW003676**
		MACN 43719$^{R/TE}$	**MW002940**	—	**MW003087**	**MW003179**	**MW003256**	**MW003352**	**MW003418**	**MW003570**	**MW003677**
R. scitula	—	IIBP 849$^{R/TE}$	**MW002941**	**MW003022**	**MW003088**	**MW003180**	**MW003257**	**MW003353**	**MW003419**	**MW003571**	**MW003678**
R. cf. *scitula*	*R. scitula*	CFBH 42359TE	—	**MW003023**	—	—	—	—	—	**MW003572#**	—
R. sclerocephala	—	MHNLS 7495TE	—	—	—	—	—	—	—	**MW003573**	—
R. spinulosa papillosa	*R. papillosa*	BB 983TE	**MW002942**	**MW003024**	**MW003089**	**MW003181**	**MW003258**	**MW003354**	**MW003420**	**MW003574**	**MW003679**
		BB 1032TE	—	—	—	—	DQ283775	—	—	DQ283046	—
		MACN 49782TE	**MW002943**	—	—	—	—	—	**MW003421**	**MW003575**	**MW003680**
		NB 96-23TE	—	—	KJ609676	KJ609676	—	—	—	AY680263	—

APPENDIX 2 *continued*

RHINELLA Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. spinulosa spinulosa	*R. spinulosa*	IDLR 3837TE	DQ306566	DQ158328	DQ158405	—	—	—	—	DQ158487	—
		MUBI 10737$^{R/TE}$	—	—	—	—	—	—	**MW003422**	**MW003576**	—
		MUBI 10770TE	**MW002944**	—	—	—	—	**MW003355**	**MW003423**	**MW003577**	**MW003681**
		nv$^{R/TE}$	**MW002945**	—	—	**MW003182**	**MW003259**	**MW003356**	**MW003424**	**MW003578**	**MW003682**
	R. altiperuviana	MACN 49701$^{R/TE}$	**MW002946**	—	**MW003090**	**MW003183**	**MW003260**	**MW003357**	**MW003425**	**MW003579**	**MW003683**
		MNCN 41989$^{R/TE}$	**MW002947**	—	—	**MW003184**	**MW003261**	**MW003358**	**MW003426**	**MW003580**	**MW003684**
R. spinulosa trifolium	*R. trifolium*	CORBIDI 5530TE	—	—	—	—	—	—	—	**MW003581#**	—
		nv^{TE}	**MW002948**	**MW003025**	**MW003091**	**MW003185**	**MW003262**	**MW003359**	—	**MW003582**	**MW003685**
R. stanlaii	—	ZUFG 6456$^{R/TE}$	**MW002949**	—	**MW003092**	**MW003186**	**MW003263**	**MW003360**	**MW003427**	**MW003583**	**MW003686**
		MNCN-ADN 4160TE	**MW002950**	—	—	—	**MW003264**	—	**MW003428**	**MW003584**	**MW003687**
		MNCN-ADN 6274TE	—	—	—	—	—	—	—	**MW003585#**	—
R. aff. *stanlaii*	*Rhinella* sp. 15	MNCN-ADN 4159TE	**MW002951**	—	—	—	—	—	—	**MW003586**	—
R. sternosignata	—	nv$^{R/TE}$	KP684943	KP685078	KP685114	KP685144	KP685163	KP685016	—	KP685184	KP685035
R. cf. *sternosignata*	*Rhinella* sp. 13	MAR 1314$^{R/TE}$	**MW002952**	**MW003026**	**MW003093**	**MW003187**	**MW003265**	**MW003361**	—	**MW003587**	**MW003688**
		MAR 1955$^{R/TE}$	**MW002953**	**MW003027**	**MW003094**	—	**MW003266**	**MW003362**	—	**MW003588**	**MW003689**
R. tacana	—	MUBI 6950$^{R/TE}$	**MW002954**	**MW003028**	**MW003095**	**MW003188**	—	**MW003363**	—	**MW003589**	**MW003690**
		MUBI 7409TE	—	—	—	—	—	—	—	**MW003590**	**MW003691**
		MUBI 7007$^{R/TE}$	**MW002955**	—	**MW003096**	**MW003189**	**MW003267**	—	—	**MW003591**	**MW003692**

APPENDIX 2 *continued*

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. tacana	—	MNK 7187$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003592#**	—
		UTA 53310$^{\mathrm{TE}}$	DQ306500	DQ158320	DQ158397	—	—	—	—	DQ158478	—
R. tenrec	—	MAR 3584$^{\mathrm{TE}}$	**MW002956**	—	—	**MW003190**	—	—	**MW003429**	**MW003593**	**MW003693**
		MAR 3585$^{\mathrm{TE}}$	**MW002957**	—	**MW003097**	—	**MW003268**	**MW003364**	**MW003430**	**MW003594#**	—
R. vellardi	—	CORBIDI 7626$^{\mathrm{TE}}$	—	—	—	—	—	—	—	**MW003595#**	—
		KU 211765$^{\mathrm{TE}}$	DQ306527	DQ158336	DQ158411	—	—	—	—	DQ158495	—
		MUBI 14281$^{\mathrm{TE}}$	**MW002958**	—	—	—	**MW003269**	—	—	**MW003596**	—
		MUBI 14291$^{\mathrm{TE}}$	**MW002959**	—	—	**MW003191**	—	—	—	**MW003597**	**MW003694**
R. veraguensis	—	IDLR 3820$^{\mathrm{TE}}$	DQ306524	DQ158337	—	—	—	—	—	DQ158496	—
		MNCN-ADN 5808$^{\mathrm{R/TE}}$	**MW002960**	—	**MW003098**	—	**MW003270**	**MW003365**	—	**MW003598**	—
		MUBI 5946$^{\mathrm{TE}}$	**MW002961**	—	**MW003099**	—	**MW003271**	—	—	**MW003599**	**MW003695**
		USNM 346048$^{\mathrm{TE}}$	DQ306535	DQ158338	—	—	—	—	—	DQ158497	—
R. veredas	—	CFBH 20516$^{\mathrm{R/TE}}$	**MW002962**	**MW003029**	**MW003100**	**MW003192**	**MW003272**	—	**MW003431**	**MW003600**	**MW003696**
		CHUNB 44609$^{\mathrm{TE}}$	**MW002963**	—	—	—	HM159245	—	HM159231	**MW003601**	**MW003697**
R. yanachaga	—	CORBIDI 7269$^{\mathrm{TE}}$	**MW002964**	—	—	—	—	**MW003366**	—	**MW003602#**	—
		MUBI 7119$^{\mathrm{R/TE}}$	**MW002965**	**MW003030**	**MW003101**	**MW003193**	**MW003273**	**MW003367**	**MW003432**	**MW003603**	**MW003698**
		MUBI 7121$^{\mathrm{TE}}$	**MW002966**	—	—	—	—	—	**MW003433**	**MW003604**	**MW003699**

APPENDIX 2 *continued*

Rhinella Current taxonomy	Updated taxonomy	Voucher	Nuclear genes						Mitochondrial genes		
			cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
R. yunga	*R. iserni*	NMP6V 75552TE	—	—	—	—	—	**MW003368**	—	**MW003605**	—
		MUSM 31096TE	**MW002967**	—	**MW003102**	—	—	**MW003369**	—	**MW003606**	—
		MUSM 31097TE	—	—	—	—	—	—	—	**MW003607**	—
		MUSM 31950TE	—	—	—	—	—	—	—	**MW003608**	—
R. sp. "gr. *acrolopha*"	*Rhinella* sp. 4	TG 2115TE	—	—	—	—	—	—	KT221613	KT221613	KT221613
R. sp. gr. *margaritifera*	*R. dapsilis*	MC 204TE	—	—	—	—	—	—	—	EF364269 EF364295	—
		PG 144TE	—	—	—	—	—	—	—	EF364276 EF364302	—
	Rhinella sp. 5	QCAZ 53072TE	—	—	**MW003103**	**MW003194**		—	—	**MW003609#**	—
		QCAZ 53142TE	—			**MW003195**		**MW003370**	—	**MW003610#**	—
	Rhinella sp. 13	MHNLS 21837TE	—	—	—	—	—	—	—	**MW003611**	—
R. sp. gr. *marina*	"*R. icterica*"	LGE 19195TE	**MW002968**	**MW003031**	**MW003104**	—	—	—	**MW003434**	**MW003612**	**MW003700**

Outgroups	Voucher	Nuclear genes						Mitochondrial genes		
		cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
Amazophrynella aff. *minuta*^{TE}	MJH 7095	—	—	—	DQ503337	AY844555	—	AY843804	DQ502120	
Anaxyrus americanus^{TE}	CAS 207258	FJ882730	—	KF666350	—	—	FJ882676	—	FJ882827	FJ882827
Anaxyrus boreas$^{R/TE}$	CAS 201586	FJ882732	—	—	—	—	FJ882678	—	FJ882830	FJ882830

APPENDIX 2 *continued*

OUTGROUPS	Voucher	Nuclear genes						Mitochondrial genes		
		cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
Anaxyrus quercicus^{TE}	MVZ 223370	—	—	KJ609658	KJ609658	KJ532269	—	—	AY680235	—
Anaxyrus woodhousii^{TE}	KU 224658	DQ306551	DQ158339	DQ158413	—	—	—	—	DQ158498	—
Ansonia longidigita^{TE}	VUB 666	FJ882698	—	KF666400	—	—	FJ882645	—	FJ882796	FJ882796
Bufo bufo^{TE}	VUB 982	FJ882704	—	—	—	—	FJ882650	—	FJ882806	FJ882806
	MVZ 230209	—	DQ158280	—	—	—	—	—	—	—
Bufo gargarizans$^{R/TE}$	CAS 228184	FJ882708	—	KF666177	—	—	FJ882654	—	FJ882808	FJ882808
	USNM 292081	—	DQ158270	—	—	—	—	—	—	—
Bufotes luristanicus^{TE}	NP 13-1	GU226833	—	—	—	—	GU226832		GU226835	GU226835
Duttaphrynus melanostictus$^{R/TE}$	VUB 52	AY364167	—	AY364197	—	AF249097	AY948805	AF249082	FJ882791	FJ882791
Incilius alvarius^{TE}	USNM 320001	DQ306516	DQ158267	DQ158351	—	—	—	—	DQ158425	—
	TWR 628	—	—	—	—	—	—	—	—	AY819460
Incilius coniferus^{TE}	KU 217480	DQ306534	DQ158286	DQ158368	—	—	—	—	DQ158445	—
Incilius nebulifer$^{R/TE}$	UTA 52489	HM563916	—	HM564002	—	—	—	HM563960	—	—
	DCC 3170	—	HQ290825	—	—	—	HQ290705		HQ290945	HQ290945
Incilius valliceps^{TE}	UTA 13097	—	—	KJ609665	KJ609665	KJ532273	—	AY008216	AY680253	—
Ingerophrynus galeatus^{TE}	FMNH 256443	DQ306506	DQ158293	DQ158374	—	—	—	—	DQ158452	—
Leptophryne borbonica^{TE}	VUB 673	EF107450	—	KF666468	—	—	EF107224	—	FJ882799	FJ882799
Melanophryniscus stelzneri^{TE}	VUB 985TE	AY948784	—	AY948927	—	—	AY948822	—	FJ882853	FJ882853
Mertensophryne loveridgei^{TE}	KMH 26653	FJ882721	—	—	—	—	FJ882666	—	FJ882820	FJ882820
	MCZ 32084	—	—	KF666463	—	—	—	—	—	—
Nannophryne variegata$^{R/TE}$	IZUA 3198	DQ306515	DQ158335	DQ158410	—	—	—	—	DQ158494	—
Nectophrynoides tornieri^{TE}	MW 1822	—	—	—	—	—	—	—	FJ882815	FJ882815
	BMNH 2005.1375	EF107490	—	EF107329	—	—	EF107270	—	—	—

APPENDIX 2 *continued*

OUTGROUPS	Voucher	Nuclear genes						Mitochondrial genes		
		cxcr4	*pomc*	*rag1*-a	*rag1*-b	*rho*	*slc8a1*	*cytb*	*12S-16S*	*nd1*
Pelophryne misera$^{R/TE}$	VUB 641	FJ882700	—	KF666300	—	—	FJ882647	—	FJ882800	FJ882800
Peltophryne empusa^{TE}	nv	JF342412	—	JF342370	—	—	—	—	—	—
	SBH 193517	—	—	—	—	—	—	KX788993	AF361695 AY028489	—
Peltophryne lemur^{TE}	AG nd	DQ306513	DQ158306	DQ158386	—	—	—	—	DQ158465	—
	SBH 190657	—	—	—	—	—	—	AY028506	—	—
Phrynoidis juxtaspera$^{R/TE}$	VUB 0649	FJ882710	—	KF666210	—	—	FJ882656	—	FJ882805	FJ882805
	FMNH 231245	—	DQ158304	—	—	—	—	—	—	—
Rentapia hosii$^{R/TE}$	VUB 0661	EF107449	—	EF107286	—	—	EF107223	—	FJ882804	FJ882804
Rhaebo ecuadorensis^{TE}	QCAZ 13234	DQ306548	DQ158296	DQ158377	—	—	—	—	DQ158455	—
Rhaebo guttatus^{TE}	LSUMZ 17418	DQ306497	DQ158300	DQ158381	—	—	—	—	DQ158459	—
Rhaebo nasicus^{TE}	ROM 20650	DQ306512	DQ158319	DQ158396	—	—	—	—	DQ158477	—
Schismaderma carens^{TE}	MW 4279	FJ882717	—	—	—	—	FJ882662	—	FJ882849	FJ882849
Sclerophrys brauni^{TE}	MW 3840	—	—	—	—	—	—	—	FJ882822	FJ882822
	BMNH 2002.350	EF107492	—	EF107331	—	—	EF107272	—	—	—
Sclerophrys garmani$^{R/TE}$	CAS 214829	DQ306547	DQ158294	KJ609679	KJ609679	KJ532279	FJ882668	—	FJ882823	FJ882823
Sclerophrys mauritanica^{TE}	NP 22-1	FJ882727	—	—	—	—	FJ882672	—	FJ882826	FJ882826
	VG 07-025	—	—	KF666227	—	—	—	—	—	—
Sclerophrys regularis$^{R/TE}$	KU 290435	DQ306523	DQ158326	DQ158404	—	—	—	—	DQ158485	—
Sclerophrys steindachneri$^{R/TE}$	CAS 214839	DQ306546	DQ158329	DQ158406	—	—	FJ882671	—	FJ882825	FJ882825
Vandijkophrynus robinsoni$^{R/TE}$	CAS 193549	—	—	—	—	—	—	—	GU183857	GU183857
	AACRG 68	KF665893	—	KF666198	—	—	—	—	—	—
Wolterstorffina parvipalmata$^{R/TE}$	DPL 5101	—	—	—	—	DQ283972	—	—	—	—
	MTSN 589	FJ882719	—	—	—	—	FJ882664	—	FJ882818	FJ882818

APPENDIX 3

List of Species Scored for the Phenotypic Dataset

Collection abbreviations are as follow: **BB,** Boris Blotto field series, to be accessioned in MACN; **CENAI,** Centro Nacional de Investigaciones Iológicas, Buenos Aires, Argentina; **CFBH,** Collection Célio F.B. Haddad, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil; **CORBIDI,** Centro de Ornitología y Biodiversidad, Lima, Peru; **ICN,** Universidad Nacional de Colombia, Instituto de Ciencias Naturales, Museo de Historia Natural, Bogotá, Colombia; **INPA,** Instituto Nacional de Pesquisas da Amazonia, Manaus, Brazil; **KU,** University of Kansas Natural History Museum, Lawrence, KS; **MACN,** Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”–CONICET, Buenos Aires, Argentina; **MAR,** Marco A. Rada field series; **MHUA,** Museo de Herpetología de la Universidad de Antioquia, Medellín, Colombia; **MNCN,** Museo Nacional de Ciencias Naturales, Madrid, Spain; **MNHN-Uy,** Museo Nacional de Historia Natural, Montevideo, Uruguay, **MNRJ,** Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil; **MTR,** Miguel Trefaut Rodrigues field series; **MUSM,** Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; **MVZ,** University of California, Museum of Vertebrate Zoology, Berkeley, CA; **MZUSP,** Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; **QCAZ,** Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Pichincha, Ecuador; **USNM,** National Museum of Natural History, Smithsonian Institution, Washington DC; **UTA,** University of Texas at Arlington, Department of Biology, Arlington, TX; **ZFMK,** Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany; **ZISP,** Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; and **ZSM,** Zoologische Staatssammlung München, München, Germany. The scoring were based on original observations or extracted/scored from literature. Abbreviations and symbols: $^{\text{Cho}}$, Larval chondrocranium; $^{\text{Ext}}$, External morphology; $^{\text{Mus}}$, Musculature; $^{\text{Ost}}$, Osteology; $^{\text{Vis}}$, Visceral anatomy; $^{\#}$, Species not included in the total evidence phylogenetic analysis but considered in the discussion; *, original observations.

Rhinella

Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. achalensis	—	MACN 24953$^{\text{Ost*}}$; Cei, 1972b$^{\text{Ext}}$, 1980$^{\text{Ext}}$	—	Vera Candioti et al., 2016	Cei, 1972b, 1980; Sinsch et al., 2001	—
R. achavali	—	MNHN-Uy 9301$^{\text{Ext*}}$; Maneyro et al., 2004$^{\text{Ext}}$; Kwet et al., 2006$^{\text{Ext}}$; Maciel et al., 2010$^{\text{Ext, Ost, Vis}}$	Kolenc et al., 2013$^{\text{Ext, Cho}}$	—	—	Kolenc et al., 2013
R. acrolopha	—	KU 76961$^{\text{Ext*}}$, 76965$^{\text{Ext*}}$; 76984$^{\text{Ost*}}$; Trueb, 1971$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	Trueb, 1971	—
R. acutirostris	—	MTR 36684 $^{\text{Ext*}}$; ZSM 1147-0$^{\text{Ext*}}$; Lötters and Köhler, 2000$^{\text{Ext}}$	—	—	Dias, personal commun.	—
R. alata	—	Liu, 1935$^{\text{Ext}}$; Vélez-Rodríguez, 2004$^{\text{Ost}}$; Santos et al., 2015$^{\text{Ext}}$	—	—	Santos et al., 2015	—
R. amabilis	*R.* cf. *amabilis*	Morrison, 1994$^{\text{Ost}}$; Pramuk and Kadivar, 2003 $^{\text{Ext, Ost}}$; Pramuk, 2006$^{\text{Ext, Ost}}$	—	—	—	—
R. amboroensis	*R. quechua*	Harvey and Smith, 1993$^{\text{Ext}}$	—	—	Harvey and Smith, 1993	—

APPENDIX 3 *continued*

RHINELLA

Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. arborescandens	—	CORBIDI 2020^{Ext*}; Duellman and Schulte, 1992	—	—	Duellman and Schulte, 1992	—
R. arenarum arenarum	*R. arenarum*	MACN 38639^{Ext*}, 39928$^{Ext*, Mus*}$, 43139 ^{Ext*}, 51116 ^{Ext*}, 51117 ^{Ext*}; MACN 53784^{Mus*}; USNM 70620^{Ost*}, 70622^{Ost*}; Cei, 1980^{Ext}; Pramuk, 2006$^{Ext, Ost}$; Silva and Mendelson, 1999^{Vis}	Fernández, 1927^{Ext}; Kehr and Williams, 1990 ^{Ext}; Vera Candioti, 2007$^{Ext, Cho}$	Vera Candioti et al., 2016	M.O.P. personal obs.; Fernández, 1927	Schmid, 1978
R. arequipensis	*R. spinulosa*	KU 14792^{Ext*}; Vellard, 1959^{Ext}; Pramuk, 2006 $^{Ext, Ost}$	Aguilar and Gamarra, 2004^{Ext}	—	—	Córdova, 1999
R. arunco	—	KU 217363^{Ost*}, 217369^{Ext*}; MZUSP 29961^{Mus*}; Cei, 1962^{Ext}; Martin, 1972a^{Ost}, 1972b^{Ost}; Silva and Mendelson, 1999^{Vis}; Pramuk, 2006$^{Ext, Ost}$; Urra, 2013 ^{Ext}	Müller and Hellmich, 1932^{Ext}	—	Urra, 2013	Formas, 1978
R. atacamensis	—	KU 217351^{Ost*}, 217352^{Ext*}; Cei, 1962^{Ext}; Martin 1972a^{Ost}, 1972b^{Ost}; Silva and Mendelson, 1999^{Vis}; Urra, 2013 ^{Ext}	Cei, 1962^{Ext}	—	Cei, 1962; Urra, 2013	—
R. azarai	—	LGE 8710 ^{Ext*}, 15163^{Ost*}, LGE 15190^{Ost*}; Gallardo, 1965^{Ext}; Narvaes and Rodrigues, 2009^{Ext}	Blotto et al., 2014^{Ext}	Vera Candioti et al., 2016	Blotto et al., 2014; Pereyra et al., 2015	—
R. beebei	—	USNM 566017^{Ost*}; Gallardo, 1965^{Ext}; Kenny, 1969^{Ext}; Pramuk, 2006$^{Ext, Ost}$; Murphy et al., 2017 ^{Ext}	Kenny, 1969^{Ext}	Nokhbatolfoghahai and Downie, 2005, 2008	Narvaes and Rodrigues, 2009	—
R. bergi	—	LGE 15180^{Ost*}; CFBH 3273^{Mus*}; MACN 50519$^{Ext*, Vis*}$, 50519^{Ext*}, 50523^{Vis*}; Céspedez, 2000^{Ext}; Narvaes and Rodrigues, 2009^{Ext}	—	—	Yanosky et al., 1997; Narvaes and Rodrigues, 2009	—
R. bernardoi	—	Sanabria et al., 2010 $^{Ext, Ost}$	Grosso et al., 2020^{Ext}	—	—	—
R. casconi	—	CFBH 22863^{Ext*}, 28175^{Ext*}; Roberto et al., 2014^{Ext}	Roberto et al., 2014^{Ext}	—	Roberto et al., 2014	—
R. castaneotica	—	USNM 518807^{Ost*}; Caldwell, 1991^{Ext}	Caldwell, 1991^{Ext}	—	Caldwell, 1991; Noronha et al., 2013	—

APPENDIX 3 *continued*

Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
RHINELLA						
R. ceratophrys	*Rhaebo ceratophrys*	Boulenger, 1882$^{\text{Ext}}$; Rodríguez and Duellman, 1994$^{\text{Ext}}$; Rojas-Runjaic et al., 2017 $^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	Rodríguez and Duellman, 1994; Fenolio et al., 2012	—
R. cerradensis	—	Maciel et al., 2007 $^{\text{Ext, Ost}}$; Maciel et al., 2010$^{\text{Ost}}$	Maciel et al., 2007$^{\text{Ext}}$	Vera Candioti et al., 2016	—	—
R. chavin	—	Lehr et al., 2001$^{\text{Ext}}$, 2005$^{\text{Ost}}$; Pramuk and Lehr, 2005$^{\text{Ost}}$; Pramuk, 2006$^{\text{Ost}}$	—	—	Lehr et al., 2001	—
R. chrysophora#	—	McCranie et al., 1989$^{\text{Ext, Ost}}$; McCranie and Wilson, 2002$^{\text{Ext}}$; Pramuk and Lehr, 2005$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; McCranie, 2017$^{\text{Ext}}$	McCranie et al., 1989$^{\text{Ext}}$; Lavilla and de Sá, 2001$^{\text{Cho}}$; Pramuk and Lehr, 2005$^{\text{Ext}}$	—	McCranie and Wilson, 2002	—
R. cristinae#	—	Vélez-R. and Ruiz-C., 2002$^{\text{Ext, Ost, Vis}}$	—	—	Vélez-R. and Ruiz-C., 2002	—
R. crucifer	—	CFBH 24629$^{\text{Mus*}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Baldissera et al., 2004$^{\text{Ext}}$; Pramuk, 2006 $^{\text{Ext, Ost}}$	Ruas et al., 2012$^{\text{Ext}}$; Oliveira et al., 2014$^{\text{Cho}}$	—	—	Baldissera et al., 1999
R. dapsilis	—	CFBH 11398$^{\text{Mus*}}$; QCAZ 17719 $^{\text{Ext*}}$; 38892$^{\text{Ext*}}$, 135$^{\text{Ext*}}$; USNM 196951$^{\text{Ost*}}$, 201814$^{\text{Ost*}}$; Myers and Carvalho, 1945$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	—	—
R. diptycha	—	CFBH 1327$^{\text{Ext*}}$, 5084$^{\text{Ext*}}$, KU 289057 $^{\text{Ext*}}$; LGE 135$^{\text{Ext*}}$; USNM 281765$^{\text{Ost*}}$; Cei, 1980$^{\text{Ext}}$; Pramuk, 2006$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	Rossa-Feres and Nomura, 2006$^{\text{Ext}}$; Oliveira et al., 2014$^{\text{Cho}}$	—	M.O.P. and D. B., personal obs.	Kasahara et al., 1996; Baldissera et al., 1999; Azevedo et al., 2003
R. dorbignyi	—	MACN 43700$^{\text{Ext*, Vis*}}$, 43701$^{\text{Ext*, Vis*}}$; Gallardo, 1965$^{\text{Ext}}$; Martin, 1972a$^{\text{Ost}}$, 1972b$^{\text{Ost}}$; Cei, 1980$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Narvaes and Rodrigues, 2009$^{\text{Ext}}$	Borteiro et al., 2006$^{\text{Ext}}$	—	—	—
R. fernandezae	*R. dorbignyi*	MACN 39383$^{\text{Ost*}}$; MACN 39345$^{\text{Mus*}}$, 39350$^{\text{Ext*, Mus*}}$, 40251$^{\text{Ext*}}$, 40257$^{\text{Ext*}}$, 40259$^{\text{Ext*}}$; Gallardo, 1965$^{\text{Ext}}$; Narvaes and Rodrigues, 2009$^{\text{Ext}}$	Fernández, 1927$^{\text{Ext}}$; Lavilla et al., 2000$^{\text{Ext}}$; Borteiro et al., 2006$^{\text{Ext}}$	Vera Candioti et al., 2016	Fernández, 1927; Pereyra et al., 2015	—

APPENDIX 3 *continued*

Rhinella

Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. festae	—	KU 217501$^{\text{Ext*}}$; USNM 167168$^{\text{Ost*}}$; Trueb, 1971$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	Trueb, 1971	—
R. gallardoi	—	CENAI 2657$^{\text{Ext*}}$, 2658$^{\text{Ext*}}$, 2882$^{\text{Ost*}}$, 3090$^{\text{Ext*}}$; Carrizo, 1992$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	—	—
R. gildae	—	Vaz-Silva et al., 2015$^{\text{Ext}}$; Ávila et al., 2018	—	—	—	—
R. gnustae#	—	MACN 4775$^{\text{Ext*}}$; Gallardo 1967	—	—	—	—
R. granulosa	—	CFBH 18706$^{\text{Ext*}}$; Gallardo, 1965$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Narvaes and Rodrigues, 2009 $^{\text{Ext}}$	Mercês et al., 2009$^{\text{Ext}}$; Oliveira et al., 2014$^{\text{Cho}}$	—	Narvaes and Rodrigues, 2009	Baldissera et al., 1999
R. henseli	—	CFBH 18238$^{\text{Ext*}}$, 20117$^{\text{Ext*}}$; CFBH 24054$^{\text{Mus*}}$; Baldissera et al., 2004$^{\text{Ext}}$	Oliveira et al., 2014$^{\text{Cho}}$	—	—	Bruschi et al., 2019
R. hoogmoedi	—	CFBH 24088$^{\text{Mus*}}$; MNRJ 40328$^{\text{Ext*}}$; Caramaschi and Pombal, 2006$^{\text{Ext}}$; Roberto et al., 2011$^{\text{Ext}}$	Merces et al., 2009$^{\text{Ext}}$; Oliveira et al., 2014$^{\text{Cho}}$	—	—	Bruschi et al., 2019
R. horribilis	—	KU 202274$^{\text{Ext*}}$, 217482$^{\text{Ext*}}$, 289750$^{\text{Ext*}}$; UTA 54882 $^{\text{Ext*}}$; Martin, 1972a$^{\text{Ost}}$, 1972b$^{\text{Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	Savage, 2002$^{\text{Ext}}$; Lynch, 2006$^{\text{Ext}}$	Breder, 1946	Breder, 1946; Guayara-Barragán and Bernal, 2012	Córdova and Descailleaux, 1996
R. humboldti	—	Gallardo, 1965$^{\text{Ext}}$; Pramuk, 2006 $^{\text{Ext, Ost}}$; Narvaes and Rodrigues, 2009$^{\text{Ext}}$; Murphy et al., 2017	Lynch, 2006$^{\text{Ext}}$	—	Narvaes and Rodrigues, 2009; Guayara-Barragan and Bernal, 2012	—
R. icterica	—	CFBH 11027$^{\text{Ext*}}$, 13965$^{\text{Ext*}}$, 27410$^{\text{Ext*}}$, 38392$^{\text{Ext*}}$; USNM 100954$^{\text{Ost*}}$, 100957$^{\text{Ost*}}$; Heyer et al., 1990$^{\text{Ext*}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Maciel et al., 2010$^{\text{Ost}}$	Heyer et al., 1990$^{\text{Ext}}$; Oliveira et al., 2014$^{\text{Cho}}$	—	Heyer et al., 1990	Baldissera et al., 1999; Azevedo et al., 2003
R. inca	—	Stejneger, 1913$^{\text{Ext}}$; Pramuk and Lehr, 2005$^{\text{Ext}}$	—	—	—	—
R. inopina	—	Vaz-Silva et al., 2012$^{\text{Ext}}$	—	—	Vaz-Silva et al., 2012	—
R. iserni#	—	MNCN 3057$^{\text{Ext*}}$; Jiménez de la Espada, 1875$^{\text{Ext}}$	—	—	—	—

APPENDIX 3 *continued*

RHINELLA

Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. jimi	*R. diptycha*	CFBH 8638*, 9310*, 19335*$^{\text{Ext}}$, 19512$^{\text{Mus*}}$; Stevaux, 2002$^{\text{Ext}}$; Maciel et al., 2010 $^{\text{Ext, Ost, Vis}}$;	Merces et al., 2009$^{\text{Ext}}$; Tolledo and Toledo, 2010$^{\text{Ext}}$; Oliveira et al., 2014$^{\text{Cho}}$	—	Stevaux, 2002	—
R. justinianoi	—	Harvey and Smith, 1994$^{\text{Ext}}$	—	—	Harvey and Smith, 1994	—
R. leptoscelis	—	Padial et al., 2009$^{\text{Ext}}$	—	—	—	—
R. lescurei	—	Fouquet et al., 2007a$^{\text{Ext}}$	—	—	Fouquet et al., 2007a	—
R. lilyrodriguezae	—	CORBIDI 3780$^{\text{Ext*}}$; Cusi et al., 2017 $^{\text{Ext}}$	—	—	—	—
R. limensis	—	Vellard, 1959$^{\text{Ext}}$; Morrison, 1994$^{\text{Ost}}$; Pramuk and Kadivar, 2003$^{\text{Ost}}$; Pramuk, 2006$^{\text{Ext; Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	Angulo and Aguilar, 2003$^{\text{Ext}}$	—	—	Cordova, 1999
R. lindae	—	MAR 3329 $^{\text{Ext*}}$, 3431$^{\text{Ext*}}$, 3432 $^{\text{Ext*}}$; Rivero and Castaño, 1990 $^{\text{Ext}}$	—	—	Rivero and Castaño, 1990	—
R. macrorhina	—	MVZ 150267$^{\text{Ext*}}$; Trueb, 1971$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	—	—
R. magnussoni	—	Lima et al., 2007$^{\text{Ext}}$	Lima et al., 2007$^{\text{Ext}}$	—	Lima et al., 2007	—
R. major	—	LGE 12146$^{\text{Ost*}}$; MACN 39100$^{\text{Mus*}}$, 50518$^{\text{Ext*}}$, 50519$^{\text{Ext*}}$, 50220$^{\text{Ext*}}$; Gallardo, 1965$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk, 2006$^{\text{Ext, Ost}}$; Narvaes and Rodrigues, 2009 $^{\text{Ext}}$	Grosso et al., 2020$^{\text{Ext}}$	—	Perotti, 1994; Pereyra et al., 2015	—
R. manu	—	Chaparro et al., 2007$^{\text{Ext, Ost}}$	—	—	Chaparro et al., 2007	—
R. margaritifera	—	ZISP 257.1$^{\text{Ext*}}$, 257.2$^{\text{Ext*}}$	—	—	—	—
R. cf. *margaritifera*	*Rhinella* sp. 10	Pramuk, 2006 (as *Bufo margaritifer* 2)$^{\text{Ext, Ost}}$;	—	—	—	—
R. cf. *margaritifera*	*Rhinella* sp. 14	KU 181623$^{\text{Ost*}}$, 215145$^{\text{Ext*}}$, 215146$^{\text{Ext*}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Vélez-Rodríguez, 2004b (as *Bufo* sp. “6”)$^{\text{Ext}}$	—	—	—	—

APPENDIX 3 *continued*

RHINELLA						
Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. marina	—	CFBH 1325*, 15711*$^{\text{Ext}}$; KU 205236*$^{\text{Ext}}$; Kenny, 1969$^{\text{Ext}}$; Pramuk, 2006$^{\text{Ext, Ost}}$	Kenny, 1969$^{\text{Ext}}$; Rada de Martínez, 1990$^{\text{Ext}}$; Duellman, 2005$^{\text{Ext}}$	—	Duellman, 2005	Baldissera et al., 1999
R. martyi	*R. margaritifera*	Fouquet et al., 2007a $^{\text{Ext}}$	—	—	Fouquet et al., 2007a	—
R. merianae	—	CFBH 16641$^{\text{Ext*}}$; MTR 20517$^{\text{Ext*}}$; USNM 566017$^{\text{Ost*}}$, 566018$^{\text{Ost*}}$; Gallardo, 1965$^{\text{Ext}}$; Narvaes and Rodrigues, 2009 $^{\text{Ext}}$	Hero, 1990$^{\text{Ext}}$; Silva et al., 2017$^{\text{Ext}}$	—	Hödl, 1990; Lima et al., 2007	—
R. mirandaribeiroi	—	CFBH 28396$^{\text{Mus*}}$; Gallardo, 1965$^{\text{Ext}}$; Pramuk, 2006$^{\text{Ext, Ost}}$; Narvaes and Rodrigues, 2009 $^{\text{Ext}}$	Grosso et al., 2020$^{\text{Ext}}$	—	Narvaes and Rodrigues, 2009	—
R. multiverrucosa	*R.* cf. *multiverrucosa*	Lehr et al., 2005$^{\text{Ext, Ost}}$; Pramuk, 2006$^{\text{Ext, Ost}}$	—	—	Lehr et al., 2005	—
R. nattereri#	—	Bokermann 1967$^{\text{Ext}}$, Narvaes and Rodrigues, 2009$^{\text{Ext}}$	—	—	—	—
R. nesiotes	—	KU 154920$^{\text{Ext*}}$; Duellman and Toft, 1979$^{\text{Ext}}$	—	—	—	—
R. nicefori	—	MHUA 4793$^{\text{Ext*}}$; Trueb, 1971$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	—	—
R. ocellata	—	CFBH 26592$^{\text{Ext}}$, 28398$^{\text{Ext, Mus*}}$; USNM 130177$^{\text{Ost*}}$; Leão and Cochran, 1952$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Matavelli et al., 2014 $^{\text{Ext}}$	—	—	Leao and Cochran, 1952; Caldwell and Shepard, 2007	—
R. ornata	—	CFBH 11061$^{\text{Ext*}}$, 12269$^{\text{Ext*}}$, 38375$^{\text{Ext*}}$; LGE 4020$^{\text{Ost*}}$, 6503$^{\text{Ext*}}$, 8729$^{\text{Ext*}}$, 15161$^{\text{Ost*}}$, 19014 $^{\text{Ext*}}$; USNM 70613$^{\text{Ost*}}$, 70614$^{\text{Ost*}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	Heyer et al., 1990$^{\text{Ext}}$; Oliveira et al., 2014$^{\text{Cho}}$	—	C.F.B.H., personal obs.; Heyer et al., 1990; Pereyra et al., 2015;	Baldissera et al., 1999
R. paraguas	—	Grant and Bolivar-G, 2014$^{\text{Ext, Ost}}$; CD 870$^{\text{Mus*}}$	—	—	Grant and Bolivar-G, 2014	—
R. paraguayensis	*R. scitula*	MACN 19040$^{\text{Ext*, Vis*}}$, 19052$^{\text{Ext*, Vis*}}$; Ávila et al., 2010 $^{\text{Ext}}$	—	—	Ávila et al., 2010	—
R. poeppigii	—	USNM 268824$^{\text{Ext*}}$, 346829$^{\text{Ost*}}$, 346830$^{\text{Ost*}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; de la Riva, 2002$^{\text{Ext}}$; León de Castro and Rey Sánchez, 2014 $^{\text{Ext}}$	—	—	de la Riva, 2002	Córdova and Descailleaux, 1996

APPENDIX 3 *continued*

RHINELLA

Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. proboscidea	—	ZSM 1145-0^{Ext*}; Lima et al., 2006^{Ext}	Menin et al., 2006^{Ext}	—	Lima et al., 2006; Menin et al., 2006; Pereyra et al., 2015	Bruschi et al., 2019
R. pygmaea	—	CFBH 2894^{Ext*}, 5006^{Mus*}; MACN 4177^{Ext*}; Gallardo, 1965^{Ext}; Silva and Mendelson, 1999^{Vis}; Narvaes and Rodrigues, 2009^{Ext}, Simon and Marroig, 2015^{Ost}	Carvalho and Carvalho, 1994^{Ext}; Oliveira et al., 2014^{Cho}	—	Carvalho and Carvalho, 1994	Baldissera et al., 1999
R. quechua	—	CENAI nv^{Ost*}; MACN 46656$^{Ext, Vis}$, 46662^{Ext*}, 46663$^{Ext, Mus*}$, 46668^{Ext*}, 46670^{Ext*}	Aguayo et al., 2009$^{Ext, Cho}$	—	Köhler, 2000, Pereyra et al., 2015	—
R. roqueana#	—	Melin, 1941^{Ext}; Hoogmoed, 1986^{Ext}; Vélez-Rodríguez, 2004$^{Ext, Ost}$	—	—	—	—
R. rostrata#	—	Trueb, 1971$^{Ext, Ost}$	—	—	Trueb, 1971	—
R. rubescens	—	CFBH 1910^{Ext*}, 2587$^{Ext, Mus*}$, 2588^{Ext*}, 4451^{Ext*}, 5836^{Ext*}; Silva and Mendelson, 1999^{Vis}; Maciel et al., 2010^{Ost};	Eterovick and Sazima, 1999^{Ext}	—	Haddad et al., 1988	—
R. rubropunctata	—	KU 159966^{Ost*}; MACN 12377^{Ext*}, 12380^{Ext*}, 15408$^{Ext*, Mus*}$, 15409^{Ext*}, 15412^{Ext*}; Cei, 1962^{Ext}, 1980^{Ext}; Silva and Mendelson, 1999^{Vis}	Formas and Pugin, 1978^{Ext}	—	—	Formas, 1978; Schmid et al., 2004
R. ruizi	—	Grant, 2000^{Ext}	—	—	Grant, 2000	—
R. rumbolli	—	CENAI 2661^{Ost*}; LGE 6441^{Ost*}; MACN 43713$^{Ext*, Mus*, Vis*}$; MACN 43719$^{Ext*, Mus*, Vis*}$; MACN 53783^{Ext*}	Haad et al., 2014$^{Ext, Cho}$	Vera Candioti et al., 2016	Pereyra et al., 2015	—
R. scitula	—	Caramaschi and Niemeyer, 2003^{Ext}	Caramaschi and Niemeyer, 2003^{Ext}	—	Caramaschi and Niemeyer, 2003	—
R. sclerocephala	—	Mijares-Urrutia and Arends, 2001^{Ext}	—	—	Mijares-Urrutia and Arends, 2001	—
R. sebbeni#	—	Vaz-Silva et al., 2015^{Ext}	—	—	—	—
R. spinulosa papillosa	*R. papillosa*	MACN 42253^{Ext*}, 42254$^{Ext*, Vis*}$, MACN 42255^{Ext*}, 49696^{Mus*}; Cei, 1980^{Ext}	Donoso-Barros, 1975^{Ext}	—	Sympson et al., 2006; Pereyra et al., 2015	—

APPENDIX 3 *continued*

Rhinella						
Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. spinulosa spinulosa	*R. spinulosa*	KU 163033$^{\text{Ost*}}$; MUSM 19376$^{\text{Ext*}}$, 19477$^{\text{Ext*}}$; Vellard, 1959$^{\text{Ext}}$; Boulenger, 1910$^{\text{Vis}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	Aguilar and Gamarra, 2004$^{\text{Ext}}$	—	—	Córdova, 1999
R. spinulosa spinulosa	*R. altiperuviana*	KU 160271$^{\text{Ost*}}$; MACN 49701$^{\text{Mus*}}$	Vera Candioti, 2007$^{\text{Ext, Cho}}$	Vera Candioti et al., 2016	Sympson et al., 2006; Urra, 2013; Pereyra et al., 2015	
R. stanlaii	—	Lötters and Köhler, 2000$^{\text{Ext}}$	—	—	Lötters and Köhler, 2000	—
R. sternosignata	—	La Marca and Mijares-Urrutia, 1996$^{\text{Ext}}$; Vélez-Rodríguez, 2005$^{\text{Ost}}$	—	—	La Marca and Mijares-Urrutia, 1996	—
R. tacana	—	Padial et al., 2006$^{\text{Ext}}$	—	—	Padial et al., 2006, J.C.C., personal obs.	—
R. tenrec	—	MAR 3584$^{\text{Ext*}}$; Lynch and Renjifo, 1990$^{\text{Ext}}$	—	—	Lynch and Renjifo, 1990	—
R. trifolium	—	Vellard, 1959$^{\text{Ext}}$	Aguilar and Gamarra, 2004$^{\text{Ext}}$	—	—	Córdova, 1999
R. truebae#	—	Lynch and Renjifo, 1990$^{\text{Ext, Ost}}$	—	—	—	—
Rhinella sp. gr. *margaritifera*	*Rhinella* sp. 5	QCAZ53072$^{\text{Ext*}}$, 53142$^{\text{Ext*}}$	—	—	—	—
R. vellardi	—	KU 211765$^{\text{Ext*}}$; Vellard, 1959$^{\text{Ext}}$; Morrison, 1994$^{\text{Ost}}$; Pramuk and Kadivar, 2003$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$	—	—	—	—
R. veraguensis	—	KU 164084$^{\text{Ost*}}$; ZFMK 80578$^{\text{Ext*}}$; Boulenger, 1882$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk and Lehr, 2005$^{\text{Ext}}$; Pramuk, 2006$^{\text{Ext, Ost}}$; Padial et al., 2009 $^{\text{Ext}}$	Cadle and Altig, 1991$^{\text{Ext}}$; Pramuk and Lehr, 2005$^{\text{Ext}}$	—	Köhler, 2000	—
R. veredas	—	Brandão et al., 2007$^{\text{Ext}}$; Maciel et al., 2010$^{\text{Ost}}$	—	—	Brandão et al., 2007	—
R. yanachaga	—	Lehr et al., 2007$^{\text{Ext, Ost}}$	—	—	Lehr et al., 2007	—

APPENDIX 3 *continued*

Rhinella

Current taxonomy	Updated taxonomy	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
R. yunga	*R. iserni*	Moravec et al., 2014$^{\text{Ext}}$; Stynoski et al., 2020$^{\text{Ext}}$	Stynoski et al., 2020$^{\text{Ext}}$	—	Stynoski et al., 2020	—

APPENDIX 3 *continued*

Outgroups	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
Amazophrynella aff. *minuta*	ICN 46770$^{\text{Mus*}}$; McDiarmid, 1971$^{\text{Ext, Ost}}$; Duellman, 1978$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Lima et al., 2006$^{\text{Ext}}$	Duellman and Lynch, 1969$^{\text{Ext}}$; Duellman, 1978$^{\text{Ext}}$	—	Lima et al., 2006	—
Anaxyrus americanus	Boulenger, 1882$^{\text{Ext}}$; Wright and Wright, 1949$^{\text{Ext}}$; McAlister, 1961$^{\text{Ext}}$; Tihen, 1962$^{\text{Ost}}$; Pramuk, 2006$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999 $^{\text{Vis}}$	Larson, 2004$^{\text{Cho}}$; Altig and McDiarmid, 2015$^{\text{Ext}}$	—	Wright and Wright, 1949; Altig and McDiarmid, 2015	Schmid, 1978
Anaxyrus boreas	Boulenger, 1882$^{\text{Ext}}$, 1910$^{\text{Vis}}$; Wright and Wright, 1949$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk, 2006$^{\text{Ext, Ost}}$	Altig and McDiarmid, 2015$^{\text{Ext}}$	—	Wright and Wright, 1949	Schmid, 1978
Anaxyrus woodhousii	MACN 42131$^{\text{Ext*,Mus*}}$, 42132$^{\text{Ext*,Mus *}}$, 42133$^{\text{Ext*}}$; McAlister, 1961$^{\text{Ext}}$; Martin, 1973$^{\text{Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk, 2006$^{\text{Ext, Ost}}$	Altig and McDiarmid, 2015$^{\text{Ext}}$	—	Wright and Wright, 1949	—
Ansonia longidigita	ZFMK 80678 $^{\text{Ext*}}$, 80689$^{\text{Ext*}}$, 80690 $^{\text{Ext*}}$, 80693$^{\text{Ext*}}$; Inger, 1960$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Malkmus et al., 2002 $^{\text{Ext}}$	Haas et al., 2009$^{\text{Ext}}$	—	Inger, 1960; Malkmus et al., 2002	—
Bufo bufo	Boulenger, 1897$^{\text{Ext, Ost}}$; Pramuk, 2006$^{\text{Ost}}$; Boulenger, 1910$^{\text{Vis}}$; Plytycz and Szarski, 1987$^{\text{Vis}}$; Pramuk, 2006 $^{\text{Ext}}$	Boulenger, 1897$^{\text{Ext}}$; Haas, 2003$^{\text{Cho}}$	Boulenger, 1897	Boulenger, 1897	Schmid, 1978; Birstein and Mazin, 1982
Duttaphrynus melanostictus	Boulenger, 1882 $^{\text{Ext}}$, 1910$^{\text{Vis}}$ van Kampen, 1923$^{\text{Ext}}$; Inger, 1966$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk, 2006 $^{\text{Ext, Ost}}$	van Kampen, 1923$^{\text{Ext}}$; Haas, 2003$^{\text{Cho}}$	—	van Kampen, 1923; Ahmad and Alam, 2015	—
Incilius alvarius	Cope, 1889$^{\text{Ext}}$; McAlister, 1961$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk, 2006 $^{\text{Ext, Ost}}$	Altig and McDiarmid, 2015$^{\text{Ext}}$	—	Wright and Wright, 1949	—

APPENDIX 3 *continued*

Outgroups	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
Incilius coniferus	USNM 348058$^{\text{Ost}*}$, 348059$^{\text{Ost}*}$; Cope 1862$^{\text{Ext}}$; Savage, 2002$^{\text{Ext}}$; Pramuk, 2006$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Mendelson et al., 2011$^{\text{Ext, Mus}}$	Savage, 2002$^{\text{Ext}}$	—	Mendelson et al., 2011	—
Incilius valliceps	Boulenger, 1882$^{\text{Ext}}$; Cope, 1889$^{\text{Ext}}$; Wright and Wright, 1949$^{\text{Ext}}$; McAlister, 1961$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk, 2006 $^{\text{Ext, Ost}}$	Savage, 2002$^{\text{Ext}}$	—	Wright and Wright, 1949	Schmid, 1978
Melanophryniscus gr. *stelzneri*	LGE 16644 (*M. klappenbachi*)$^{\text{Mus}*}$; MACN 47819 (*M.* cf. *estebani*)$^{\text{Ext}}$; MACN 49593 (*M. klappenbachi*)$^{\text{Mus}*}$; McDiarmid, 1971 (*M. stelzneri*)$^{\text{Ext, Ost}}$; Pramuk, 2006 (*M. fulvoguttatus*)$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999 (*M. stelzneri*)$^{\text{Vis}}$	Fernández, 1927 (*M. stelzneri*)$^{\text{Ext}}$; Baldo et al., 2014 (*M. stelzneri*) $^{\text{Ext, Cho}}$	Vera Candioti et al., 2016 (*M. klappenbachi* and *M. stelzneri*)	Fernández, 1927 (*M. stelzneri*)	Baldo et al., 2012 (*M. stelzneri*)
Nannophryne variegata	BB 2336$^{\text{Mus}*}$; MACN 41447$^{\text{Ext}*,\ \text{Vis}*}$, 45175$^{\text{Ext}*,\ \text{Mus}*,\ \text{Vis}*}$, 41452$^{\text{Ost}*}$, 52417$^{\text{Ext}*,\ \text{Mus}*,\ \text{Vis}*}$; USNM 15124 $^{\text{Ost}*}$; Pramuk, 2006 $^{\text{Ost}}$	Donoso-Barros, 1975$^{\text{Ext}}$; Formas and Pugin 1978$^{\text{Ext}}$	—	Hock, 1967; Formas and Pugin, 1978; Cei, 1980	Formas, 1978
Peltophryne empusa	MACN 39143$^{\text{Ext}*;\ \text{Mus}*}$; MACN 39145$^{\text{Ext}*;\ \text{Mus}*}$; Liu, 1935$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Díaz and Cádiz, 2008$^{\text{Ext}}$	Díaz and Cádiz, 2008 $^{\text{Ext}}$	—	Díaz and Cádiz, 2008	—
Peltophryne lemur	Pramuk, 2006$^{\text{Ext, Ost}}$; Díaz-Lameiro et al., 2010$^{\text{Ext}}$	Rivero et al., 1980$^{\text{Ext}}$	—	Rivero et al., 1980; Lentini, 2000	—
Phrynoidis juxtaspera	Pramuk, 2006$^{\text{Ext, Ost}}$; Silva and Mendelson, 1999 $^{\text{Vis}}$; Inger, 1966$^{\text{Ext}}$; Malkmus, 2002$^{\text{Ext}}$	Malkmus, 2002$^{\text{Ext}}$	—	Malkmus, 2002	—
Rentapia hosii	Pramuk, 2006 $^{\text{Ext, Ost}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Boulenger, 1892$^{\text{Ext}}$; van Kampen, 1923$^{\text{Ext}}$; Inger, 1966$^{\text{Ext}}$	Inger, 1985$^{\text{Ext}}$; Haas, 2003$^{\text{Cho}}$	—	van Kampen, 1923	Schmid, 1978
Rhaebo guttatus	Pramuk, 2006$^{\text{Ext, Ost}}$; INPA 15647$^{\text{Mus}*}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Duellman, 2005$^{\text{Ext}}$	Duellman, 2005$^{\text{Ext}}$	—	Duellman, 2005; Kok and Kalamandeen, 2008	—
Schismaderma carens	CENAI 6007$^{\text{Ext, Mus}*}$; USNM 153377$^{\text{Ost}*}$, 153380$^{\text{Ost}*}$; Boulenger, 1910$^{\text{Vis}}$; Poynton, 1964$^{\text{Ext}}$; Stewart, 1967$^{\text{Ext}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Pramuk, 2006$^{\text{Ost, Ext}}$	Power, 1925$^{\text{Ext}}$; Channing et al., 2012$^{\text{Ext}}$; Viertel and Channing, 2017$^{\text{Ext}}$	—	Rose, 1962; Balinsky, 1969; Harper et al., 2010	—

APPENDIX 3 *continued*

OUTGROUPS	Adult morpology	Tadpole morphology	Embryonic morphology	Natural history	Cytogenetics
Sclerophrys mauritanica	USNM 346809$^{\text{Ost*}}$, 346811$^{\text{Ost*}}$; Boulenger, 1880$^{\text{Ost, Ext}}$, 1910$^{\text{Vis}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Liu, 1935$^{\text{Ext}}$; Beukema et al., 2013 $^{\text{Ext}}$	Beukema et al., 2013$^{\text{Ext}}$	—	Beukema et al., 2013	Schmid, 1978
Sclerophrys regularis	Pramuk, 2006$^{\text{Ost, Ext}}$; MZUSP 148117$^{\text{Mus*}}$; Boulenger, 1910$^{\text{Vis}}$; Silva and Mendelson, 1999$^{\text{Vis}}$; Boulenger, 1880$^{\text{Ext}}$; Liu, 1935$^{\text{Ext}}$; Stewart, 1967$^{\text{Ext}}$	Sedra, 1950 $^{\text{Cho}}$; Sedra and Michael, 1958$^{\text{Cho}}$; Channing et al., 2012$^{\text{Ext}}$	—	Stewart, 1967	—
Vandijkophrynus robinsoni	Branch and Braack, 1995$^{\text{Ext}}$	Branch and Braack, 1995$^{\text{Ext}}$; Channing et al., 2012$^{\text{Ext}}$	—	Branch and Braack, 2004	—

APPENDIX 4

List of Studied Specimens for the Phenotypic Dataset

Museum specimens used to score the character states reported in appendix 3. Species are listed following the taxonomic changes implemented in this study (synonyms between quotation marks). See appendix 3 for institutional codes details. Abbreviations: C&S, cleared and stained specimen; μCT, Tridimensional osteological reconstructions of the specimen; DSk, dry skeleton; F, female; M, male; SA, subadult; nd, not determined.

Rhinella

Current taxonomy	Updated taxonomy	Acronym	Locality	Sex	Observations
R. achalensis	—	MACN 24953	Argentina: Córdoba: San Alberto, Pampa de Achala	nd	C&S
R. achavali	—	MNHN-Uy 9301	Uruguay: Treinta y Tres: Quebrada de los Cuervos	nd	—
R. acrolopha	—	KU 76961	Panama: Darién: Cerro Mali	SA	—
		KU 76965	Panama: Darién: Cerro Mali	M	—
		KU 76984	Colombia: Chocó: N slope Cerro Mali	SA	C&S
R. acutirostris	—	MTR 36684	Brazil: Amazonas: Comunidade Indígena Caiuá, Rio Içá	M	—
		ZSM 1147-0	Brazil: "flumen Amazonum"	M	holotype
R. arborescandens	—	CORBIDI 2020	Peru: Amazonas: Bagua	nd	—
R. arenarum arenarum	*R. arenarum*	MACN 38639	Argentina: San Luis: Ayacucho	F	—
		MACN 39928	Argentina: Ciudad de Buenos Aires	F	—
		MACN 43139	Argentina: San Luis: Junín, Santa Rosa de Conlara	M	—
		MACN 51116	Argentina: San Luis: Pringles, near La Carolina	nd	—
		MACN 51117	Argentina: San Luis: Pringles, near La Carolina	nd	—
		MACN 53784	Argentina: Salta: Santa Victoria, Quebrada "El Lapachar"	M	—
		USNM 70620	Uruguay: Montevideo: Montevideo	nd	μCT (Morphosource ID 22592)
		USNM 70622	Uruguay: Montevideo: Montevideo	nd	μCT (Morphosource ID 22593)
R. arequipensis	*R. spinulosa*	KU 14792	Peru: Arequipa: Zamacola, Cerro Colorado	F	—
R. arunco	—	KU 217363	Chile: Santiago: 2 km S Rungue	SA	C&S
		KU 217369	Chile: Santiago: 2 km S Rungue	SA	—
		MZUSP 29961	Chile: Santiago: Santiago	F	—
R. atacamensis	—	KU 217351	Chile: Coquimbo: Cuesta Pajonales, 117 km N La Serena	nd	C&S
		KU 217352	Chile: Coquimbo: Cuesta Pajonales, 117 km N La Serena	SA	—

APPENDIX 4 *continued*

Rhinella

Current taxonomy	Updated taxonomy	Acronym	Locality	Sex	Observations
R. azarai	—	LGE 15163	Argentina: Misiones: Capital: Villa Lanús, Campus Universidad Nacional de Misiones	F	C&S
		LGE 15190	Argentina: Misiones: Capital: Villa Lanús, Campus Universidad Nacional de Misiones	nd	C&S
		LGE 8710	Argentina: Misiones: Candelaria	nd	—
R. beebei	—	USNM 566017	Guyana: East Berbice: Dubulay Ranch on the Berbice River	nd	C&S
R. bergi	—	LGE 15180	Argentina: Santa Fe: General Obligado, Ruta 32, 13 km S Villa Ana	M	—
		CFBH 3273	Brazil: Mato Grosso do Sul: Corumbá, Passo da Lontra	M	—
R. casconi	—	CFBH 22863	Brazil: Ceará: Guaramiranga	F	
		CFBH 28175	Brazil: Ceará: Guaramiranga	M	holotype
R. castaneotica	—	USNM 518807	Brazil: Pará: Itaituba: Río Tapajos ca.65 km SW of Parque Nacional da Amazônia	nd	C&S
R. cerradensis	—	CHUNB 38670	Brazil: Bahia: Cocos	M	—
		CHUNB 38671	Brazil: Bahia: Cocos	M	—
R. crucifer	—	CFBH 24629	Brazil: Bahia: Camacan, Serra Bonita	M	—
R. dapsilis	—	CFBH 11398	Tocantins: Babaçulândia: Eixo Ferrovia Norte-Sul – Brejinho	F	—
		QCAZ 17719	Ecuador: Napo: Cando	M	—
		QCAZ 38892	Comunidad Kurintza: Campo Villano	F	—
		QCAZ 43967	Ecuador: Orellana: Parque Nacional Yasuní, Comunidad Añangu, Río Napo	nd	—
		USNM 196951	Ecuador: Pastaza: Río Rutuno, tributario del Río Bobonaza	nd	C&S
		USNM 201814	Brazil: Amazonas: Borba, Rio Madeira	nd	µCT (Morphosource ID 23326)
R. diptycha	—	CFBH 1327	Brazil: São Paulo: Rio Claro	M	—
		CFBH 5084	Brazil: São Paulo: Rio Claro	M	—
		KU 289057	Paraguay: Concepción: Parque Nacional Serranía San Luis	F	—
		LGE 135	Argentina: Santiago del Estero: Ojo de Agua	M	—
		USNM 281765	Bolivia: Santa Cruz: Santa Cruz	nd	µCT (Morphosource ID 23334)
R. dorbignyi	—	MACN 43700	Argentina: Buenos Aires: Dolores	M	—
		MACN 43701	Argentina: Buenos Aires: Dolores	M	—

APPENDIX 4 *continued*

Rhinella					
Current taxonomy	Updated taxonomy	Acronym	Locality	Sex	Observations
R. fernandezae	*R. dorbignyi*	MACN 40251	Argentina: Chaco: Resistencia, Peaje General Belgrano, Antequera	M	—
		MACN 40257	Argentina: Chaco: Resistencia, Peaje General Belgrano, Antequera	M	—
		MACN 40259	Argentina: Chaco: Resistencia, Peaje General Belgrano, Antequera	F	—
		MACN 39345	Entre Ríos: Islas del Ibicuy	M	—
		MACN 39350	Entre Ríos: Islas del Ibicuy	nd	—
		MACN 39383	Entre Ríos: Islas del Ibicuy	F	C&S
R. festae	—	USNM 167168	Ecuador: Pastaza: Alto Río Pucayacu	nd	C&S
		KU 217501	Locación Petrolera Garza 1, NE Montalvo	F	—
R. gallardoi	—	CENAI 2657	Argentina: Jujuy: Calilegua, Monolito	M	holotype
		CENAI 2658	Argentina: Jujuy: Calilegua, Monolito	F	—
		CENAI 2882	Argentina: Jujuy: Calilegua, Monolito	nd	DSk
		CENAI 3090	Argentina: Jujuy: Calilegua, Monolito	F	—
R. gnustae#	—	MACN 4775	Argentina: Jujuy: Rio Grande	SA	holotype
R. granulosa	—	CFBH 18706	Brazil: Espírito Santo: Linhares	nd	—
R. henseli	—	CFBH 18238	Brazil: Rio Grande do Sul: Bento Gonçalves	nd	—
		CFBH 20117	Brazil: Rio Grande do Sul: Catiporã	nd	—
		CFBH 24054	Brazil: Paraná: São Mateus do Sul	M	—
R. hoogmoedi	—	CFBH 24088	Brazil: São Paulo: Peruíbe, Trilha 11	M	—
		MNRJ 40328	Brazil: Bahia: Canavieiras	F	paratype
R. horribilis	—	KU 289750	El Salvador: Ahuachapan: Parque Nacional El Imposible, La Fincona	F	—
		UTA 54882	Mexico: Veracruz: Veracruz	F	—
	Rhinella sp. 1	KU 202274	Ecuador: Pichincha: Tinalandia, 15.5 km SE Santo Domingo de los Colorados	M	—
		KU 217482	Ecuador: Loja: Vilcabamba	M	—
R. icterica	—	CFBH 11027	Brazil: Santa Catarina: Bom Jardim da Serra	M	—
		CFBH 13965	Brazil: Rio de Janeiro: Petrópolis	M	—
		CFBH 27410	Brazil: Rio de Janeiro: Município de Cachoeiras de Macacu, Parque Estadual dos Três Picos	M	—
		CFBH 38392	Brazil: Rio de Janeiro	F	—
		USNM 100954	Brazil: São Paulo: São Paulo, Parque Jabaquara	nd	µCT (Morphosource ID 23329)
		USNM 100957	Brazil: São Paulo: São Paulo: Parque Jabaquara	nd	µCT (Morphosource ID 23330)
R. iserni	—	MNCN 3057	Peru: Junin: N.E. Tarma, Andes de Chanchamayo	F	holotype

APPENDIX 4 *continued*

RHINELLA

Current taxonomy	Updated taxonomy	Acronym	Locality	Sex	Observations
R. jimi	*R. diptycha*	CFBH 8638	Brazil: Pernambuco: Fernando de Noronha	M	—
		CFBH 9310	Brazil: Pernambuco: Fernando de Noronha	M	—
		CFBH 19335	Brazil: Bahia, Maracás: Fazenda Cana Brava	F	—
		CFBH 19512	Brazil: Bahia, Maracás: Fazenda Cana Brava	M	—
R. lilyrodriguezae	—	CORBIDI 3780	Peru: Huancabamba	F	—
R. lindae	—	MAR 3329	Colombia: Antioquia: Parque Nacional Natural Las Orquídeas	nd	—
		MAR 3431	Colombia: Antioquia: Parque Nacional Natural Las Orquídeas	M	—
		MAR 3432	Colombia: Antioquia: Parque Nacional Natural Las Orquídeas	nd	—
R. macrorhina#	—	MVZ 150267	Colombia: Antioquia: 0.5 km W (by road) Medellin	nd	—
R. major	—	LGE 12146	Argentina: Chaco: General Güemes, near Wichi	M	C&S
		MACN 39100	Argentina: Salta: near Dragones	M	—
R. cf. *margaritifera*	*Rhinella* sp. 14	KU 181623	Peru: Amazonas: near Balsapata	nd	C&S
		KU 215145	Peru: Madre de Dios: Cuzco Amazónico, 15 km E Puerto Maldonado	F	—
		KU 215146	Peru: Madre de Dios: Cuzco Amazónico, 15 km E Puerto Maldonado	F	—
R. margaritifera	—	ZISP257.1	"Brasilia"	F	lectotype
		ZISP257.2	"Brasilia"	F	paralectotype
R. marina	—	CFBH 1325	Brazil: Pará: Paraopebas	M	—
		CFBH 15711	Brazil: Acre: Tarauacá	nd	—
		KU 205236	Peru: Madre de Dios: Cuzco Amazónico, 15 km E Puerto Maldonado	M	—
R. merianae	—	CFBH 16641	Brazil: Amazonas: Manaus, Reserva Duke	nd	—
		MTR 20517	Brazil: Roraima: Estação Ecológica de Maracá	nd	—
		USNM 566017	Guyana: East Berbice: Dubulay Ranch	nd	µCT (Morphosource ID 23327)
		USNM 566018	Guyana: East Berbice: Dubulay Ranch	nd	µCT (Morphosource ID 23328)
R. mirandaribeiroi	—	CFBH 28396	Brazil: Tocantins: Porto Nacional	M	—
R. nesiotes	—	KU 154920	Peru: Huanuco: W slope Serrania de Sira	F	holotype
R. nicefori	*R.* cf. *nicefori*	MHUA 4793	Colombia: Antioquía: Belmira	nd	—
R. ocellata	—	CFBH 26592	Brazil: Maranhão: Barreirinhas	nd	—
		CFBH 28398	Brazil: Tocantins: Porto Nacional	F	—
		USNM 130177	Brazil: Goiás: Rio Araguaia, between Santa Leopoldina and Ilha do Bananal	nd	µCT (Morphosource ID 23331)

APPENDIX 4 *continued*

Rhinella

Current taxonomy	Updated taxonomy	Acronym	Locality	Sex	Observations
R. ornata	—	CFBH 11061	Brazil: Paraná: Antonina, Trilha do Ferro	nd	—
		CFBH 12269	Brazil: São Paulo: Caraguatatuba	nd	—
		CFBH 38375	Brazil: Rio de Janeiro: Visconde de Mauá	nd	—
		LGE 15161	Argentina: Misiones: Cainguás, Aristóbulo del Valle, Arroyo Cuñá Pirú Chico	M	C&S
		LGE 19014	Argentina: Misiones: Iguazú, Parque Provincial Uruguaí	M	—
		LGE 4020	Argentina: Misiones	nd	C&S
		LGE 6503	Argentina: Misiones: Cainguás, Aristóbulo del Valle, Arroyo Cuñá Pirú Chico		—
		LGE 8729	Argentina: Misiones: Capital, Profundidad, Parque Provincial Profundidad	nd	—
		USNM 70613	Brazil: Rio de Janeiro: Rio de Janeiro	F	µCT (Morphosource ID 22596)
		USNM 70614	Brazil: Rio de Janeiro: Rio de Janeiro	F	µCT (Morphosource ID 22597)
R. paraguas	—	CD 870	Colombia: Valle del Cauca: Dagua	M	—
R. paraguayensis	*R. scitula*	MACN 19040	Bolivia: Santa Cruz: Santa Cruz de la Sierra, Surutú, Río Colorado	nd	—
		MACN 19052	Bolivia: Santa Cruz: Santa Cruz de la Sierra, Surutú, Río Colorado	nd	—
R. poeppigii	—	USNM 346829	Peru: San Martín: Aucayacu, Río Huallaga	nd	µCT (Morphosource ID 23332)
		USNM 346830	Peru: San Martín: Aucayacu, Río Huallaga	nd	µCT (Morphosource ID 23333)
R. pygmaea	—	CFBH 2894	Brazil: Bahia: Ilhéus	nd	—
		CFBH 5006	Brazil: Rio de Janeiro: São João da Barra	M	—
		MACN 4177	Brazil: Rio de Janeiro: São João da Barra	nd	paratype
R. quechua	—	CENAI ND	Bolivia: Carrasco: Cochabamba	SA	DSk
		MACN 46656	Bolivia: Carrasco: Cochabamba	F	—
		MACN 46662	Bolivia: Carrasco: Cochabamba	M	—
		MACN 46663	Bolivia: Carrasco: Cochabamba	M	—
		MACN 46668	Bolivia: Carrasco: Cochabamba	M.	—
		MACN 46670	Bolivia: Carrasco: Cochabamba	M.	—
R. rubescens	—	CFBH 2587	Brazil: Distrito Federal: Brasília	M	—
		CFBH 1910	Brazil: Distrito Federal: Brasília	M	—
		CFBH 2587	Brazil: Distrito Federal: Brasília	M	—
		CFBH 2588	Brazil: Distrito Federal: Brasília	M	—
		CFBH 4451	Brazil: Minas Gerais: Morro do Ferro, Poços de Caldas	F	—

APPENDIX 4 *continued*

Rhinella

Current taxonomy	Updated taxonomy	Acronym	Locality	Sex	Observations
R. rubescens	—	CFBH 5836	Brazil: Minas Gerais: Morro do Ferro, Poços de Caldas	SA	—
R. rubropunctata	—	KU 159966	Chile, Llanquihue: Lago Todos Los Santos	nd	C&S
		MACN 15408	Argentina: Río Negro: Bariloche, El Bolsón	F	
		MACN 12377	Argentina: Río Negro: Bariloche, El Bolsón	F	—
		MACN 12380	Argentina: Río Negro: Bariloche, El Bolsón	M	—
		MACN 15409	Argentina: Río Negro: Bariloche, El Bolsón	F	—
		MACN 15412	Argentina: Río Negro: Bariloche, El Bolsón	M	—
R. rumbolli	—	CENAI 2661	Argentina: Jujuy: Calilegua, Monolito	nd	DSk
		LGE 6441	Argentina: Jujuy: Doctor Manuel Belgrano, Ocloyas	M	C&S
		MACN 43713	Argentina: Salta: Santa Victoria, Parque Nacional Baritú	M	—
		MACN 43719	Argentina: Salta: Santa Victoria, Parque Nacional Baritú	M	—
		MACN 53783	Argentina: Salta: Santa Victoria, Parque Nacional Baritú	F	—
R. spinulosa papillosa	*R. papillosa*	MACN 49696	Argentina: Rio Negro: Bariloche	M	—
		MACN 42253	Argentina: Río Negro: Bariloche, Pampa Linda, near Cerro Tronador	F	—
		MACN 42254	Argentina: Río Negro: Bariloche, Pampa Linda, near Cerro Tronador	M	—
		MACN 42255	Argentina: Río Negro: Bariloche, Pampa Linda, near Cerro Tronador	M	—
R. spinulosa spinulosa	*R. spinulosa*	KU 163033	Peru: Puno: 4 km W Santa Rosa	nd	C&S
		MUSM 19376	Peru: Puno	F	—
		MUSM 19477	Peru: Puno	M	—
	R. altiperuviana	MACN 49701	Argentina: Jujuy: Quebrada de Sepultura	M	—
R. tenrec	—	MAR 3584	Colombia: Antioquia: Parque Nacional Natural Las Orquídeas	nd	—
Rhinella sp. gr. *margaritifera*	*Rhinella* sp. 5	QCAZ 53072	Ecuador: Pastaza: Montalvo	M	—
		QCAZ 53142	Ecuador: Pastaza: Montalvo	F	—
R. vellardi	—	KU 211765	Peru: Cajamarca: Cajabamba, 10 km SSE Cajabamba	nd	—
R. veraguensis		ZFMK 80578	Bolivia: La Paz: between Caranavi and Palos Blancos	M	—
	—	KU 164084	Peru: Cusco: 4 km SW Santa Isabel, Rio Cosnipata	M	DSk

APPENDIX 4 *continued*

Outgroups	Acronym	Locality	Sex	Observations
Amazophrynella aff. *minuta*	ICN 46770	Colombia: Amazonas: Leticia	M	—
Anaxyrus woodhousii	MACN 42131	ND (Pet trade)	M	—
	MACN 42132	ND (Pet trade)	M	—
	MACN 42133	ND (Pet trade)	M	—
Ansonia longidigita	ZFMK 80678	Malaysia: Sabah: Mt. Kinabalu	M	—
	ZFMK 80689	Malaysia: Sabah: Mt. Kinabalu	M	—
	ZFMK 80690	Malaysia: Sabah: Mt. Kinabalu	M	—
	ZFMK 80693	Malaysia: Sabah: Mt. Kinabalu	M	—
Incilius coniferus	USNM 348058	Panama: Bocas del Toro: Isla Cristobal	F	µCT (Morphosource ID 21896)
	USNM 348059	Panama: Bocas del Toro: Isla Cristobal	F	µCT (Morphosource ID 21897)
Melanophryniscus stelzneri group	LGE 16644 (*M. klappenbachi*)	Argentina: Chaco: San Fernando, Club Sixty Resistencia	nd	—
	MACN 47819 (*M.* cf. *estebani*)	Argentina: San Luis: Libertador General San Martín, Dique La Huertita	M	—
	MACN 49593 (*M. klappenbachi*)	Argentina: Chaco: San Fernando, Club Sixty Resistencia	nd	—
Nannophryne variegata	MACN 52417	Argentina: Santa Cruz: Lago Argentino, Lago del Desierto	M	—
	BB 2336	Argentina: Santa Cruz: Lago Argentino, Lago del Desierto	nd	—
	MACN 41452	Argentina: Santa Cruz: Lago Argentino, Lago del Desierto	M	C&S
	MACN 41475	Argentina: Santa Cruz: Lago Argentino, Lago del Desierto	nd	—
	USNM 15124	Chile: Magallanes: Mayne Harbor	nd	µCT (Morphosource ID 21909)
Peltophryne empusa	MACN 39143	Cuba: Isla de la Juventud: 14.7 mi al SSW de Nueva Gerona	M	—
	MACN 39145	Cuba: Isla de la Juventud: 14,7 mi al SSW de Nueva Gerona	M	—
Rhaebo guttatus	INPA 15647	Brazil: Amazonas	nd	—
Schismaderma carens	CENAI 6007	nd	SA	—
	USNM 153377	Malawi: Rumphi: Rumph	nd	µCT (Morphosource ID 23335)
	USNM 153380	Malawi: Rumphi: Rumph	nd	µCT (Morphosource ID 23336)
Sclerophrys mauritanica	USNM 346809	Morocco: Tetouan: 20 km SE Larache	nd	µCT (Morphosource ID 21854)
	USNM 346811	Morocco: Tetouan: 20 km SE Larache	nd	µCT (Morphosource ID 21855)
Sclerophrys regularis	MZUSP 148117	Guiné Bissau: Beli, Boé	M	—

APPENDIX 5

Assignation of the Species of *Rhinella* to Species Groups by Different Authors

Assignations provided in the original description of the species are indicated with an asterisk (*).

Species	Original description	Species group assigned
R. abei	Baldissera et al., 2004 (as *Bufo abei*)	*R. crucifer* Group*
R. achalensis	Cei, 1972b (as *Bufo achalensis*)	*R. spinulosa* Group*
R. achavali	Maneyro et al., 2004 (as *Bufo achavali*)	*R. marina* Group*
R. acrolopha	Trueb, 1971 (as *Rhamphophryne acrolopha*)	*R. acrolopha* Group (Grant and Bolivar-G., 2014)
R. acutirostris	Spix, 1824 (as *Bufo acutirostris*)	*R. margaritifera* Group (Hoogmoed, 1986)
R. alata	Thominot, 1884 (as *Bufo alatus*)	*R. margaritifera* Group (Cei, 1972b)
R. amabilis	Pramuk and Kadivar, 2003 (as *Bufo amabilis*)	*R. spinulosa* Group*
R. amboroensis	Harvey and Smith, 1993 (as *Bufo amboroensis*)	*R. veraguensis* Group*
R. arborescandens	Duellman and Schulte, 1992 (as *Bufo arborescandens*)	*R. veraguensis* Group*
R. arenarum	Hensel, 1867 (as *Bufo arenarum*)	*R. marina* Group (Martin, 1972b); *R. arenarum* Group (Cei, 1980); *R. marina* Group (Duellman and Schulte, 1992)
R. arequipensis	Vellard, 1959 (as *Bufo spinulosus arequipensis*)	*R. spinulosa* Group*
R. arunco	Molina, 1782 (as *Rana arunco*)	*R. spinulosa* Group (Martin, 1972b)
R. atacamensis	Cei, 1962 (as *Bufo spinulosus atacamensis*)	*R. spinulosa* Group*
R. azarai	Gallardo, 1965 (as *Bufo granulosus azarai*)	*R. granulosa* Group (Cei, 1972b)
R. beebei	Gallardo, 1965 (as *Bufo granulosus beebei*)	*R. granulosa* Group (Cei, 1972b)
R. bergi	Céspedez, 2000 (as *Bufo bergi*)	*R. granulosa* Group*
R. bernardoi	Sanabria et al., 2010	*R. granulosa* Group*
R. casconi	Roberto et al., 2014	*R. crucifer* Group*
R. castaneotica	Caldwell, 1991 (as *Bufo castaneoticus*)	*R. margaritifera* Group*
R. centralis	Narvaes and Rodrigues, 2009	*R. granulosa* Group*
R. ceratophrys	Boulenger, 1882 (as *Bufo ceratophrys*)	*R. margaritifera* Group (Cei, 1972b); unnassigned (Pramuk, 2006)
R. cerradensis	Maciel et al., 2007	*R. marina* Group*
R. chavin	Lehr et al., 2001 (as *Bufo chavin*)	*R. veraguensis* Group*; *R. festae* Group (Moravec et al. 2014)
R. chrysophora	McCranie et al., 1989 (as *Atelophryniscus chrysophorus*)	*R. veraguensis* Group (Pramuk and Lehr, 2005)
R. cristinae	Vélez and Ruiz, 2002 (as *Bufo cristinae*)	*R. margaritifera* Group (Pramuk 2006); unassigned (Fouquet et al., 2007a)
R. crucifer	Wied, 1821 (as *Bufo crucifer*)	*R. crucifer* Group (Martin, 1972b)
R. dapsilis	Myers and Carvalho, 1945 (as *Bufo dapsilis*)	*R. margaritifera* Group*
R. diptycha	Cope, 1862 (as *Bufo diptychus*)	*R. diptycha* Group (Vellard, 1959); unassigned (Duellman and Schulte, 1992); *R. marina* Group (Lavilla and Brusquetti, 2018)
R. dorbignyi	Duméril and Bibron, 1841 (as *Bufo dorbignyi*)	*R. granulosa* Group (Cei, 1972b; Martin, 1972b)
R. fernandezae	Gallardo, 1957 (as *Bufo granulosus fernandezae*)	*R. granulosa* Group (Cei, 1972b; Martin, 1972b)

APPENDIX 5 *continued*

Species	Original description	Species group assigned
R. festae	Peracca, 1904 (as *Atelopus festae*)	*R. acrolopha* Group (Grant and Bolivar-G.); *R. festae* Group (Moravec et al., 2014)
R. fissipes	Boulenger, 1903 (as *Bufo fissipes*)	*R. marina* Group (Vellard, 1959); *R. margaritifera* Group (Cei, 1972b), *R. veraguensis* Group (Hoogmoed, 1990)
R. gallardoi	Carrizo, 1992 (as *Bufo gallardoi*)	*R. veraguensis-margaritifera* Group*
R. gildae	Vaz-Silva et al., 2015	*R. margaritifera* Group*
R. gnustae	Gallardo, 1967 (as *Bufo gnustae*)	Unassigned (Duellman and Schulte, 1992)
R. granulosa	Spix, 1824 (as *Bufo granulosus*)	*R. granulosa* Group (Cei, 1972b)
R. henseli	Lutz, 1934 (as *Bufo crucifer henseli*)	*R. crucifer* Group (Baldissera et al. 2004)
R. hoogmoedi	Caramaschi and Pombal, 2006	*R. margaritifera* Group*
R. horribilis	Wiegmann, 1833 (as *Bufo horribilis*)	*R. marina* Group (Acevedo et al., 2016)
R. humboldti	Gallardo, 1965 (as *Bufo granulosus humboldti*)	*R. granulosa* Group (Cei, 1972b; Martin, 1972b)
R. icterica	Spix, 1824 (as *Bufo ictericus*)	*R. marina* Group (Martin, 1972b)
R. inca	Stejneger, 1913 (as *Bufo inca*)	*R. margaritifera* Group (Vellard, 1959); *R. veraguensis* Group (Gallardo, 1961), *R. margaritifera* Group (Cei, 1972b); *R. veraguensis* group (Hoogmoed, 1990)
R. inopina	Vaz-Silva et al., 2012	*R. crucifer* Group*
R. iserni	Jiménez de la Espada, 1875 (as *Oxyrhynchus iserni*)	*R. margaritifera* Group (Hoogmoed, 1986); unassigned (Fouquet et al., 2007a)
R. jimi	Stevaux, 2002 (as *Bufo jimi*)	*R. marina* Group*
R. justinianoi	Harvey and Smith, 1994 (as *Bufo justinianoi*)	*R. veraguensis* Group*
R. leptoscelis	Boulenger, 1912 (as *Bufo leptoscelis*)	*R. margaritifera* Group (Vellard, 1959); *R. veraguensis* Group (Gallardo 1961); *R. margaritifera* Group (Cei, 1972b); *R. veraguensis* Group (Hoogmoed, 1990)
R. lescurei	Fouquet et al., 2007a	*R. margaritifera* Group*
R. lilyrodriguezae	Cusi et al., 2017	*R. festae* Group*
R. limensis	Werner, 1901 (as *Bufo limensis*)	*R. spinulosa* Group (Vellard, 1959)
R. lindae	Rivero and Castaño, 1990 (as *Rhamphophryne lindae*)	*R. acrolopha* Group (Grant and Bolivar, 2014)
R. macrorhina	Trueb, 1971 (as *Rhamphophryne macrorhina*)	*R. acrolopha* Group (Grant and Bolivar-G., 2014); *R. festae* Group (Moravec et al., 2014)
R. magnussoni	Lima et al., 2007	*R. margaritifera* Group*
R. major	Müller and Hellmich, 1936 (as *Bufo granulosus major*)	*R. granulosa* Group (Martin, 1972b)
R. manu	Chaparro et al., 2007	*R. festae* Group (Moravec et al., 2014)
R. margaritifera	Laurenti, 1768 (as *Rana margaritifera*)	*R. margaritifera* Group (Vellard, 1959)
R. marina	Linnaeus, 1758 (as *Rana marina*)	*R. marina* Group (Vellard, 1959)
R. martyi	Fouquet et al., 2007a	*R. margaritifera* Group*
R. merianae	Gallardo, 1965 (as *Bufo granulosus merianae*)	*R. granulosa* Group (Cei, 1972b)
R. mirandaribeiroi	Gallardo, 1965 (as *Bufo granulosus mirandaribeiroi*)	*R. granulosa* Group (Cei, 1972b)
R. multiverrucosa	Lehr et al., 2005 (as *Bufo multiverrucosus*)	*R. veraguensis* Group*

APPENDIX 5 *continued*

Species	Original description	Species group assigned
R. nattereri	Bokermann, 1967 (as *Bufo granulosus nattereri*)	*R. granulosa* Group (Cei, 1972b)
R. nesiotes	Duellman and Toft, 1979 (as *Bufo nesiotes*)	*R. veraguensis* Group*; *R. festae* Group (Moravec et al., 2014)
R. nicefori	Cochran and Goin, 1970 (as *Bufo rostratus nicefori*)	*R. acrolopha* Group (Grant and Bolivar-G., 2014)
R. ocellata	Günther, 1858b (as *Bufo ocellatus*)	*R. margaritifera* Group (Cei, 1972b); unassigned Hoogmoed, 1990); *R. margaritifera* Group (Lavilla et al., 2013)
R. ornata	Spix, 1824 (as *Bufo ornatus*)	*R. crucifer* Group*
R. paraguas	Grant and Bolívar-G., 2014	*R. acrolopha* Group*
R. paraguayensis	Ávila et al., 2010	*R. margaritifera* Group*
R. poeppigii	Tschudi, 1845 (as *Bufo poeppigii*)	*R. marina* Group (Vellard, 1959)
R. proboscidea	Spix, 1824 (as *Bufo proboscideus*)	*R. margaritifera* Group (Hoogmoed, 1986)
R. pygmaea	Myers and Carvalho, 1952 (as *Bufo pygmaeus*)	*R. granulosa* Group (Cei, 1972b)
R. quechua	Gallardo, 1961 (as *Bufo quechua*)	*R. veraguensis* Group*; *R. margaritifera* Group (Cei, 1972b); *R. veraguensis* Group (Hoogmoed, 1990)
R. roqueana	Melin, 1941 (as *Bufo typhonius roqueanus*)	*R. margaritifera* Group (Hoogmoed, 1986)
R. rostrata	Noble, 1920 (as *Bufo rostratus*)	*R. acrolopha* Group (Grant and Bolivar-G., 2014); *R. festae* Group (Moravec et al., 2014)
R. rubescens	Lutz, 1925 (as *Bufo rubescens*)	*R. marina* Group (Cei, 1972b); *R. arenarum* Group (Cei, 1980)
R. rubropunctata	Guichenot, 1848 (as *Bufo rubropunctatus*)	*R. spinulosa* Group (Cei, 1972b)
R. ruizi	Grant, 2000 (as *Rhamphophryne ruizi*)	*R. acrolopha* (Grant and Bolivar-G., 2014)
R. rumbolli	Carrizo, 1992 (as *Bufo rumbolli*)	*R. veraguensis* Group*
R. scitula	Caramaschi and Niemeyer, 2003 (as *Bufo scitulus*)	*R. margaritifera* Group*
R. sclerocephala	Mijares and Arends, 2001 (as *Bufo sclerocephalus*)	*R. margaritifera* Group*
R. sebbeni	Vaz-Silva et al., 2015	*R. margaritifera* Group*
R. spinulosa	Wiegmann, 1834 (as *Bufo spinulosus*)	*R. spinulosa* Group (Vellard, 1959)
R. stanlaii	Lötters and Köhler, 2000 (as *Bufo stanlaii*)	*R. margaritifera* Group*
R. sternosignata	Günther, 1858b (as *Bufo sternosignatus*)	*R. margaritifera* Group (Cei, 1972b); unassigned (Hoogmoed, 1990); *R. margaritifera* Group (Duellman and Schulte, 1992); unassigned (Fouquet et al., 2007a)
R. tacana	Padial et al., 2006 (as *Chaunus tacana*)	*R. veraguensis* Group*
R. tenrec	Lynch and Renjifo, 1990 (as *Rhamphophryne tenrec*)	*R. acrolopha* (Grant and Bolivar-G., 2014)
R. truebae	Lynch and Renjifo, 1990 (as *Rhamphophryne truebae*)	*R. acrolopha* (Grant and Bolivar-G., 2014)
R. vellardi	Leviton and Duellman, 1978 (as *Bufo vellardi*)	*R. spinulosa* Group*
R. veraguensis	Schmidt, 1857 (as *Bufo veraguensis*)	*R. margaritifera* Group (Vellard, 1959); *R. veraguensis* Group (Gallardo, 1961); *R. margaritifera* Group (Cei, 1972b); *R. veraguensis* Group (Hoogmoed, 1990)

APPENDIX 5 *continued*

Species	Original description	Species group assigned
R. veredas	Brandão et al., 2007 (as *Chaunus veredas*)	*R. marina* Group*
R. yanachaga	Lehr et al., 2007	*R. veraguensis* Group*; *R. festae* Group (Moravec et al., 2014)
R. yunga	Moravec et al., 2014	*R. margaritifera* Group*

NOTE ADDED IN PROOF

Two new species of the *Rhinella margaritifera* Group were described while this publication was in the proofing stage: *R. parecis* Ávila et al., 2020, from Brazil and *R. exostosica* Ferrão et al., 2020, from Bolivia, Brazil, and Peru. The inclusion of the available 16S sequence (KDQF01003635, voucher specimen MTR 25730) of *R. parecis* in our TE dataset and the parsimony analysis in TNT found this specimen as the sister taxon to *Rhinella* sp. 12 (although with low support; JAF = 51%). *Rhinella exostosica* corresponds to *Rhinella* sp. 14, as our study and that of Ferrão et al. (2020) included some GenBank sequences in common from three terminals (KU 215145–6 and NMP6V 74915).

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Ferrão, M., A.P. Lima, S.R. Ron, S.P.L. dos Santos, and J. Hanken. 2020. New species of leaf-litter toad of the *Rhinella margaritifera* species group (Anura: Bufonidae) from Amazonia. Copeia 108: 967–986.