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Phylogenetic analysis of Ceratophryidae (Anura: Hyloidea) including extant and extinct species

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The Neotropical frog family Ceratophryidae is composed of wide-mouthed frogs with stout bodies. Living species of the family are consistently recovered as a monophyletic group, but with disparities among analyses regarding internal relationships. Ceratophryidae presents one of the richest fossil records in Anura. Nevertheless, phylogenetic analyses including both extant and extinct species are still scarce, and the position of fossils is persistently debated. In this sense, the systematics of the family has changed considerably in the last decade with the exclusion of *Baurubatrachus pricei* (Late Cretaceous), *Beelzebufo ampinga* (Late Cretaceous) and *Wawelia gerholdi* (early Miocene). Herein, a morphologically based phylogeny for Ceratophryidae, including living species (11 spp.) and fossil specimens (10 spp.), is used as a background to discuss the evolutionary history of the family and its classification. We phylogenetically placed *Baurubatrachus pricei*, *Beelzebufo ampinga* and *Wawelia gerholdi* as non-ceratophryids. We recovered a monophyletic Ceratophryidae: *Lepidobatrachus* and *Ceratophrys* form a clade, with *Chacophrys* as its sister group. Our analysis corroborates the *C. cornuta* and *C. aurita* groups. Among fossils, *L. australis* and *C. sagani* were recovered as valid species based on autapomorphies, and *C. rusconii* was found to be the sister of all *Ceratophrys*. *Ceratophrys ensenadensis*, *C. ameghinorum*, *C. aurita* NHMUK PV OR18895/6 and *C. sagani* belong to the *C. aurita* group. We also discuss homoplasies in Ceratophryidae, divergence-time estimates, and the evolution of ploidy and a dorsal shield in the family.

Keywords: Lissamphibia; palaeontology; systematics; taxonomy

Introduction

Ceratophryidae is a well-supported clade (Frost *et al.* 2006; Fabrezi & Quinzio 2008; Pyron & Wiens 2011; Faivovich *et al.* 2014; Jetz & Pyron 2018; Streicher *et al.* 2018) including large, robust, wide-mouthed frogs, with stout bodies and a hyperossified skeleton resulting from peramorphic heterochrony (Fabrezi 2006). The family (*sensu* Faivovich *et al.* 2014; Frost 2021) contains the genera *Ceratophrys* Wied-Neuwied, 1822 (eight spp.), *Chacophrys* Reig & Limeses, 1963 (one sp.) and *Lepidobatrachus* Budgett, 1899 (three spp.). *Ceratophrys* is the most speciose, comprising two species groups: the *cornuta* group, containing *C. calcarata* Boulenger, 1890, *C. cornuta* (Linnaeus, 1758), *C. stolzmanni* Steindachner, 1882 and *C. testudo* Andersson, 1945; and the *C. aurita* group, containing *C. aurita* (Raddi, 1823), *C. cranwelli* Barrio, 1980, *C. joazeirensis* Mercadal, 1986 and *C. ornata* (Bell, 1843) (J. D. Lynch 1982; Faivovich *et al.* 2014).

The family is usually recovered within Neobatrachia, most frequently within Hyloidea, but with unstable sister relationships. Ceratophryidae has been recovered as sister to the group including Pelodyridae, Phyllomedusidae and Hylidae (Haas 2003); sister to Telmatobiidae (Wiens *et al.* 2005; Faivovich *et al.* 2014; Sabbag *et al.* 2018); sister to all other Neobatrachia (Fabrezi 2006); sister to Batrachylini (Frost *et al.* 2006); sister to Telmatobiinae (Grant *et al.* 2006); sister to Hylidae (Roelants *et al.* 2007; Streicher *et al.* 2018; Hime *et al.* 2020); sister to Odontophrynidae (Fabrezi & Quinzio 2008; Gómez & Turazzini 2021); sister to a large clade within Hyloidea (Pyron & Wiens 2011); sister to the clade composed of Telmatobiidae and Alsodidae (Zhang *et al.* 2013); and sister to the clade Hylidae plus Hemiphractidae (Feng *et al.* 2017).

Relationships within the family have been extensively studied, based on phenotypic characters of adults (J. D. Lynch 1982; Peri 1994; Wild 1997; Fabrezi 2006;

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Fabrezi & Quinzio 2008; Vieira 2012), adults and larvae (J. D. Lynch 1982; Wild 1997; Vieira 2012), immunological data (Maxson & Ruibal 1988) and molecular markers (Faivovich *et al.* 2014). The family is consistently recovered as monophyletic, but the internal relationships are not firmly established, and all three generic arrangements have been recovered: *Ceratophrys* as sister to the clade *Chacophrys* plus *Lepidobatrachus* (Maxson & Ruibal 1988; Peri 1994; Fabrezi 2006; Faivovich *et al.* 2014; Frazão *et al.* 2015; Hutter *et al.* 2017; Brusquetti *et al.* 2018; Hime *et al.* 2020); *Chacophrys* as sister to the clade *Lepidobatrachus* plus *Ceratophrys* (J. D. Lynch 1982; Wild 1997; Fabrezi & Quinzio 2008; Faivovich *et al.* 2014; Gómez & Turazzini 2021); and *Lepidobatrachus* as sister to the clade composed of *Chacophrys* and *Ceratophrys* (Frost *et al.* 2006; Grant *et al.* 2006; Vieira 2012).

Ceratophryidae has a rich fossil record, ranging from the late Miocene (11–5 million years [Ma]) to the late Pleistocene (11,000 years), with nearly 40 fossil specimens recognized (see Nicoli 2019; Barcelos *et al.* 2020; Gómez and Turazzini 2021). Six of them are determined to the species level: *Ceratophrys ameghinorum* Fernicola, 2001; *C. ensenadensis* Rusconi, 1932; *C. prisca* Ameghino, 1899; *C. rusconii* Agnolin, 2005; *Lepidobatrachus australis* Nicoli, 2015; and, most recently, *Ceratophrys sagani* Barcelos, Almeida-Silva, Santos & Verdade, 2020. Seven of the specimens are fossil representatives of extant species: *Ceratophrys ornata* (Reig 1958; Vergnaud-Grazzini 1968; Peri 1993a; Pardiñas 2001; Pérez-Ben *et al.* 2019) and *C. aurita* (Günther 1859; Barcelos *et al.* 2020). More than 30 specimens are still not determined, currently assigned only to generic status (*Ceratophrys* or *Lepidobatrachus*). Species exclusively known from fossils from the Late Cretaceous (*Baurubatrachus pricei* Báez & Peri, 1989 and *Beelzebufo ampinga* S. E. Evans *et al.* 2008) and early Miocene (*Wawelia gerholdi* Casamiquela, 1963) once attributed to the family were recently doubted as ceratophryids (e.g. Agnolin 2012; Faivovich *et al.* 2014; Nicoli *et al.* 2016; Báez & Gómez 2018). These three fossil species were proposed as related to Calyptocephallidae, a taxon that also includes frogs with hyperossified skulls (Agnolin 2012; Nicoli *et al.* 2016; Báez & Gómez 2018). Currently, the oldest known ceratophryid fossil is *Ceratophrys* sp. MD-CH-06-165 from the upper Miocene of the Arroyo Chasicó Formation, Buenos Aires Province, Argentina (Nicoli *et al.* 2017).

The number of fossil specimens known for Ceratophryidae and the low number of specimens determined to species level, as well as a few inaccurate identifications, have motivated many studies on the

classification of ceratophryid fossils (e.g. Fernicola 2001; Nicoli 2015, 2016, 2017, 2019; Nicoli *et al.* 2016, 2017; Báez & Gómez 2018). Nevertheless, the fossil representatives of Ceratophryidae were included in a phylogeny together with the extant ones only recently (Gómez & Turazzini 2021). Phylogenetic analyses including fossils may improve the accuracy of phylogenies and increase the number of resolved nodes, regardless of the phylogenetic inference method (Gómez 2016; Koch & Parry 2020; Koch *et al.* 2021). The study of fossils can contribute to the proposition of new characters and revision of old ones, bringing light to homologous structures and revealing relationships between living groups (Patterson 1981; Marjanović & Laurin 2019). It is especially interesting to discuss two emblematic characters present among ceratophryids: polyploidy and the dorsal shield. Polyploidization events, when not disrupting or impeding development, would be advantageous by increasing genotypic variation, and have been discussed as an important speciation driver among eukaryotes (Ohno 1970; Prince & Pickett 2002; Woodhouse *et al.* 2009; Peng 2019). The dorsal shield is one of the integumentary calcifications in vertebrates (Trueb 1973), and evolved independently in many anuran taxa (i.e. Alytidae, Brachycephalidae, Bufonidae, Ceratophryidae, Dendrobatidae, Hylidae, Leptodactylidae, Microhylidae, Pelobatidae, Rhacophoridae and Ranidae; Quinzio & Fabrezi 2012 and references therein). It is usually associated with protection against desiccation and with water balance (Elkan 1968; Toledo & Jared 1993), and understanding the evolution of this character may shed light on the palaeoenvironment during ceratophryid evolution.

The goals of this paper are to: (1) propose a phylogeny including extant species and fossil specimens taxonomically assigned to Ceratophryidae; (2) re-assess the phylogeny of the clade using morphological characters; (3) use a phylogenetic analysis to assert the position of extinct species within Ceratophryidae first proposed based on overall similarities; and (4) discuss the phylogenetic affinities of *Baurubatrachus pricei*, *Beelzebufo ampinga* and *Wawelia gerholdi*. We also discuss homoplasies in Ceratophryidae, divergence-time estimates, and the evolution of ploidy and the occurrence of a dorsal shield in the family.

Material and methods

As most fossils are known only from preserved skulls, we primarily used cranial characters, but a few postcranial characters were also included. Characters were constructed upon direct examination of specimens from

museum collections (e.g. fossils, dry skeletons, alizarin-red- cleared and stained specimens), examination of micro-computed tomographic (μ CT)-scanning images taken from voucher specimens, images from Morphosource (<http://morphosource.org/>), and radiographs. We checked the species identity of all specimens scanned using the literature and comparisons to museum collections (Supplemental Material 1).

We studied one or more specimens from each extant species of Ceratophryidae (except for *Ceratophrys testudo*), and seven fossil specimens, i.e. *Ceratophrys ameghinorum*, *C. aurita* NHMUK PV OR18895/6 (*C. cornuta* of Günther 1859), *C. ensenadensis*, *C. ornata* (Vergnaud-Grazzini 1968, here referred as *Ceratophrys* sp. MNHN UN (unnumbered specimen from Muséum national d'Histoire naturelle), *C. rusconii*, *C. sagani* and *Lepidobatrachus australis*. We also included *Beelzebufo ampinga* S. E. Evans *et al.* 2008, *Baurubatrachus pricei* Báez & Peri 1989 and *Wawelia gerholdi* Casamiquela 1963, formerly attributed to Ceratophryidae (S. E. Evans *et al.* 2008, 2014; Báez *et al.* 2009). Specimens were examined under a Zeiss Stemi V11 stereomicroscope at the Universidade Federal do ABC (UFABC), and other available devices in visits to the collections of the Museo de Ciencias Naturales de La Plata (MLP) and the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN). *Lepidobatrachus australis* was coded using photographs available in Tomassini *et al.* (2011) and Nicoli (2015), and *Ceratophrys aurita* NHMUK PV OR18895/6 was coded using photographs available in Nicoli (2019) and others provided by the Natural History Museum, London (NHMUK). We supplemented our first-hand observations with descriptions and figures from the literature (Supplemental Material 1).

The Laboratory of Computerized Tomography at the Museu de Zoologia da USP (MZUSP) provided some of the μ CT-scanned specimens (Supplemental Material 1). The μ CT-scan images were prepared in a Phoenix v|tome|x m microfocus μ CT scanner Version 2.3.0.1032 (General Electric Company, Wunstorf, DE; voltage = 85 kV, current = 170 μ A). Parameters of the resulting μ CT-scan images were: pixel size 0.2, voxel size 0.04521586, resolution 96 dpi and number of images 2500. All specimens were scanned using a tungsten target, a background medium of air and no filter and were rendered as 16-bit TIFFs. The μ CT-scan images were analysed in 3DSlicer software, version 4.10.1 (Fedorov *et al.* 2012).

We used Mesquite software, version 3.6 (Maddison & Maddison 2018) to generate the character data matrix, and performed the tree analysis using new technology software (TNT version 1.5; Goloboff *et al.* 2008;

Goloboff & Catalano 2016) to run the phylogenetic analysis. In cases of an unknown character state (e.g. when a particular element is not preserved in the fossil specimen) or logical inconsistency, characters were coded as unknown. We performed a traditional search for random addition sequences with a 'random seed' value of 1, 10,000 replications, and 10 cladograms saved per replication. We used 'tree bisection and reconnection' (TBR) as the branch-swapping algorithm, collapsing trees after search. Our characters were unordered (Caetano-Anollés *et al.* 2018). We estimated parsimony jackknife absolute frequencies (Farris *et al.* 1996), using new technology (1000 replicates) and we used 10,000 replicates in TNT to calculate Bremer support (Bremer 1994). We also calculated consistency (CI; Kluge & Farris 1969) and retention (RI; Farris 1989) indices in TNT.

Phylogenetic analysis was performed using a data matrix of 99 morphological characters, including characters from J. D. Lynch (1982), Peri (1994), Wild (1997), Fabrezi & Quinzio (2008), Nicoli (2015) and Báez & Gómez (2018), sometimes rephrased following Sereno (2007), and 33 new characters. Our data matrix includes 18 terminals in the ingroup (11 extant species, five extinct ones and two fossil representatives of extant species). Additionally, 15 terminals are in the outgroup based on the results presented by J. D. Lynch (1971), Haas (2003), Wiens *et al.* (2005), Frost *et al.* (2006), Roelants *et al.* (2007), Zhang *et al.* (2013), Faivovich *et al.* (2014), Feng *et al.* (2017), Báez & Gómez (2018); Jetz & Pyron (2018), Sabbag *et al.* (2018), Streicher *et al.* (2018) and Hime *et al.* (2020): *Alsodes nodosus* (Alsodidae), *Baurubatrachus pricei* (extinct species), *Beelzebufo ampinga* (extinct species), *Calyptocephalella gayi*, *Telmatobufo venustus*, *Wawelia gerholdi* (extinct species) (Calyptocephalellidae), *Cycloramphus asper* (Cycloramphidae), *Fritziana fissilis* (Hemiphractidae), *Pseudis paradoxa* (Hylidae), *Hylodes asper* (Hylodidae), *Telmatobius degener* and *Te. thompsoni* (Telmatobiidae). The phylogenetic trees were rooted on *Calyptocephalella gayi*. We provide a list of characters with comments in Supplemental Material 1, and character 61 is figured in Supplemental Material 2. A Nexus file of our matrix is available as Supplemental Material 3. We applied parsimony ancestral state reconstruction for characters 69 (ploidy) and 73 (dorsal shield) in the software R (R Development Core Team 2021), using the package Claddis 0.6.6 (Lloyd 2016). We discuss time of divergence based on the literature (e.g. Nicoli 2015; Nicoli *et al.* 2017).

Notes on species not included

***Ceratophrys testudo* Andersson, 1945.** Andersson (1945) proposed that this extant species was closely

related to *C. calcarata*. J. D. Lynch (1982) synonymized it with *C. cornuta*. Mercadal (1988) resurrected *C. testudo* based on morphological, morphometric and cytological differences. Peri (1993b), based on a broad comparative scope, proposed that the holotype of *C. testudo* is a *C. cornuta* juvenile specimen. We only had access to an X-ray and lateral-view images of the holotype, housed in the Naturhistoriska Riksmuseet Stockholm (Swedish Museum of Natural History), Sweden. As the character coding based on the available images was not accurate, we excluded the species from our analysis.

***Ceratophrys prisca* Ameghino, 1889.** This extinct species was described by Ameghino (1899) from Monte Hermoso, Buenos Aires, Argentina. Fernicola (2001) revised the specimens of Rovereto and gave *species inquirenda* (species of doubtful identity) status to the holotype specimen of Ameghino (1899), which is currently lost (Fernicola 2001; Nicoli 2014, 2019).

***Ceratophrys prisca* var. *subcornuta* MACN 14319 and 14323.** Rovereto (1914) described and illustrated the specimen MACN 14319. Fernicola (2001) revised Rovereto's fossils and included new specimens in the sample (e.g. MACN 14325), assigning them all to *C. ameghinorum*. We verified through direct observation that the specimen MACN 14319 is deformed, possibly a product of taphonomic artefact. The deformation was not mentioned in Rovereto (1914) or in Fernicola (2001). Hence, we preferred not to include morphometric measurements or bone shape observations obtained from this specimen for further comparisons. The specimen MACN 14323 is a highly fragmented skull that was grossly restored and was never described or illustrated. We do not agree with the classification of this fossil by Mercadal de Barrio & Barrio (2002) because of differences in the skull bones of MACN 14323 compared to all other specimens assigned to *Ceratophrys prisca* var. *subcornuta* (i.e. MACN 14319 and MACN 14325), e.g. smooth medial portion of the nasals. The specimen was proposed as *Ceratophrys* sp. (Nicoli 2019). We suggest that MACN 14323 should be re-assessed.

Institutional abbreviations

FML, Fundación Miguel Lillo, Argentina; **KU**, Kansas University, USA; **MACN**, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Argentina; **MD-CH**, Arroyo Chasicó collection, Museo Municipal de Ciencias Naturales 'Carlos Darwin', Argentina; **MLP**, Museo de La Plata, Argentina; **MNHN**, Muséum national d'Histoire naturelle, France; **MZUSP**, Museu de Zoologia da Universidade de São Paulo, Brazil; **NHMUK**, Natural History Museum, London, UK; **PVL**,

Colección Paleontología Vertebrados del Instituto 'Miguel Lillo', Argentina; **SMNH**, Swedish Museum of Natural History, Sweden; **UFABC**, Universidade Federal do ABC, Brazil; **UFFRJ**, Universidade Federal Rural do Rio de Janeiro, Brazil; **UNESP**, Universidade Estadual Paulista 'Júlio de Mesquita Filho', Brazil; **USNM**, Smithsonian National Museum of Natural History, USA; **YPM**, Yale Peabody Museum, USA.

Results

Phylogenetic analysis

Our phylogenetic analysis resulted in six equally short cladograms with 273 steps, 30 terminals and 99 characters. Nodes in the outgroup and within the Ceratophryidae were mostly resolved, and the synapomorphies of the clades are listed below. The strict consensus summarizes our results (Fig. 1; CI = 0.476; RI = 0.737).

We recovered Ceratophryidae as monophyletic, supported by the following four synapomorphies (Bremer support 2; 93% jackknife): teeth condition, non-pedicellate (56-1); maxilla, pars facialis in the orbital region decreases abruptly in height (63-1); parasphenoid, total length of the alae, reduced (77-0); clavicle nearly straight shaped (82-1). The family is recovered as sister to a clade that comprises *Baurubatrachus pricei* and *Beelzebufo ampinga* (Bremer = 2). This clade [Ceratophryidae + *Ba. pricei* and *Be. ampinga*] is sister to a clade composed of *Telmatobufo* and representative taxa of Telmatobiidae, Hemiphractidae, Alsodidae, Hylidae, Hylodidae and Cycloramphidae. We also recovered *Ceratophrys* and *Lepidobatrachus* as monophyletic, and *Chacophrys* (supported by autapomorphic characters) as the sister to the clade [*Lepidobatrachus* plus *Ceratophrys*]. The autapomorphies that support *Chacophrys* are: nasals, partially covered by exostosis (character 2-1); cultriform process of parasphenoid reaching planum antorbitale (character 30-0); occipital condyles with a contiguous medial articulation (character 49-1); premaxillae with divergent alary process in frontal view (character 57-1); absence of expansion of transverse process in vertebra IV (character 87-0); complete ossification of pubis, reaching acetabular portion (character 89-2). Synapomorphies supporting *Ceratophrys* as the sister clade to *Lepidobatrachus* (Bremer = 2; 77% jackknife) are: zygomatic ramus of squamosal in contact with the maxilla and nasal (character 22-2); otic ramus of squamosal expanded and overlapping the prootic (character 23-2); otoccipital, edges of the supraterygoid fenestra mineralized (character 47-1); orbital flange of pars facialis of maxillae not

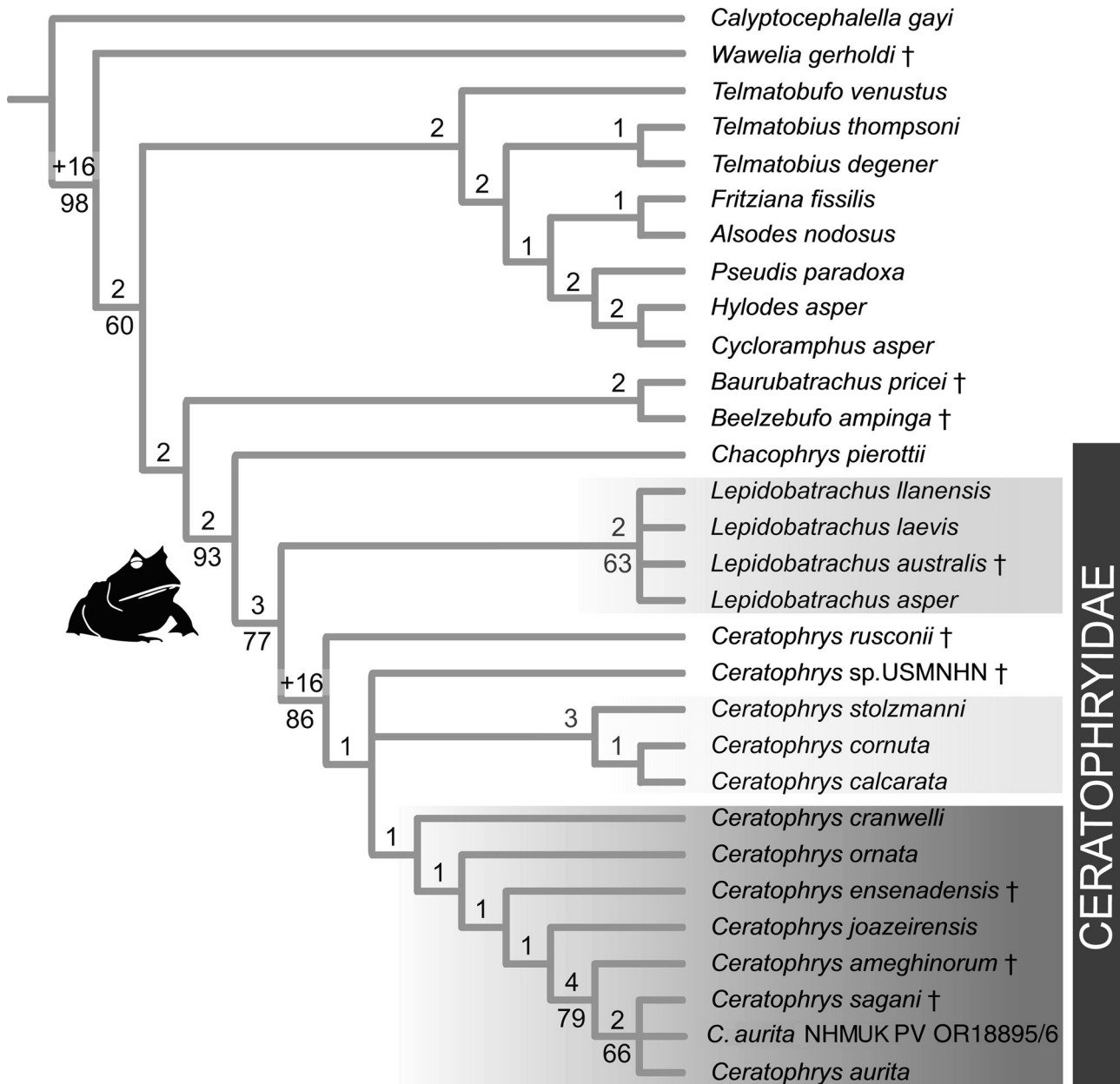


Figure 1. Strict consensus of six most parsimonious trees from the analysis using 30 terminals and a matrix of 99 characters. Bremer support values are indicated above nodes; values below nodes are parsimony jackknife frequencies. Nodes lacking values have < 50% jackknife frequencies. The three major phylogenetic groups within Ceratophryidae are shown within coloured blocks: *C. aurita* group (dark grey box), *C. cornuta* group (light grey box), and *Lepidobatrachus* genus (grey box).

participating in the formation of the orbit (character 62-1); maxilla and quadratojugal fused (character 65-1); post-temporal fenestrae present (character 66-1); ossification of the posteromedial process of hyoid invading the hyoid plate (character 79-1); and transverse process of vertebra III well expanded (character 86-2).

Relationships among species of *Lepidobatrachus* were not resolved, but the clade (Bremer = 2; 63% jackknife) is well supported by nine synapomorphies: skull bones not forming a single akinetic unit (character 3-0); wide squamosal and maxilla, (character 17-1); one vomerine tooth (character 34-1); palatine angled anterolaterally in relation to skull longitudinal axis (character 40-1);

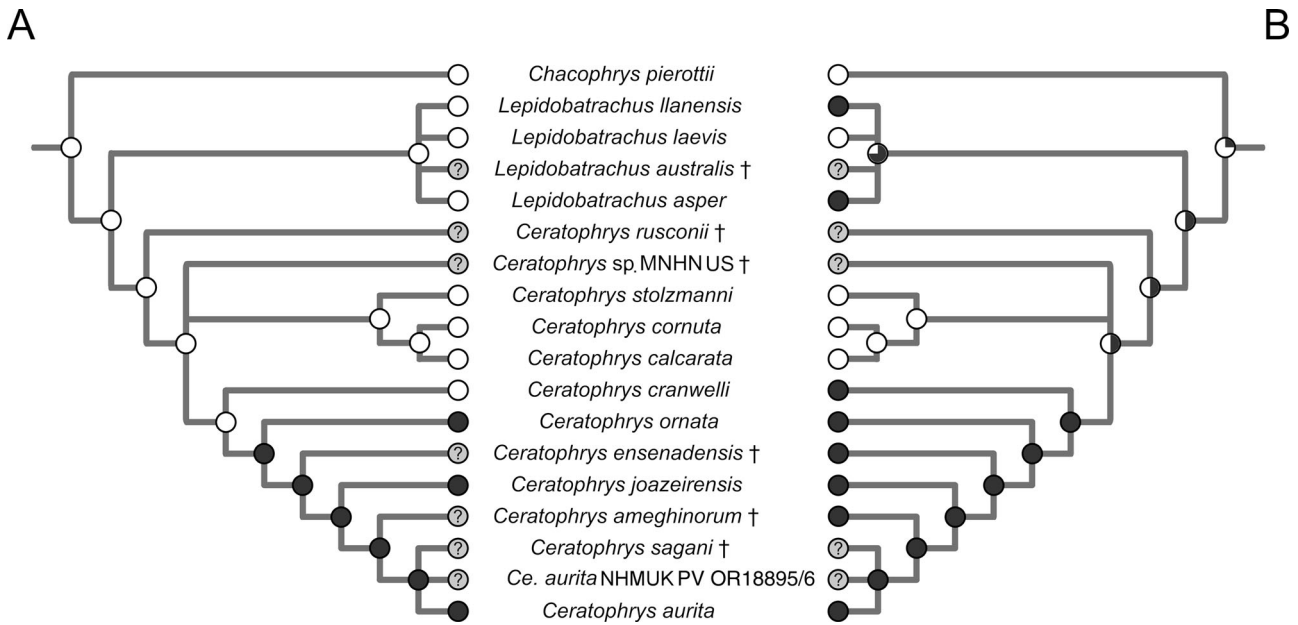


Figure 2. Optimization of ceratophryid characters on the strict consensus tree. **A**, ploidy (character 69); **B**, dorsal shield (character 73). Optimization for ploidy (grey = octaploid, white = diploid) and dorsal shield (grey = presence, white = absence) based on parsimony. The clade circles represent the most parsimonious ancestral states. Light grey circles with question marks denote unknown states.

footplate with a concave/globous ornamentation (character 53-0); long alary process of premaxilla (character 58-1); sloping dorso posteriorly (character 60-1); quadrate located well posterior relative to occipital condyle (character 67-3); and the presence of dermal ossification on parahyoid (character 80-1). Divergences among the most parsimonious trees are: *L. asper* and *L. llanensis* as sister species, or *L. laevis* as the sister of all *Lepidobatrachus*.

Ceratophrys was recovered as monophyletic (Bremer >16; 86% jackknife) supported by: anterior margin of orbits posterior to skull mid-length (character 5-1); supraorbital flange of frontoparietals angled dorsally (character 13-1); frontoparietal, postorbital and interorbital portions about the same width (character 14-1); frontoparietal and squamosal contact anteriorly to crista parotica (character 16-1); squamosal, otic plate extending posteriorly at about the same level as the occipital condyles (character 25-1); ventral process of anterior ramus of pterygoid present (character 31-1); presence of odontoids or ridges on ventral surface of palatine (character 39-1); and crista parotica ventrally positioned in relation to the dorsal edge of epiotic eminences (character 45-1). *Ceratophrys rusconii* is the sister taxon of all species of *Ceratophrys*. The relationships among the crown clade of *Ceratophrys* species are almost fully resolved. The synapomorphies supporting the *C. cornuta* group (Bremer = 3) (*C. stolzmanni*, *C. cornuta* and *C.*

calcarata) are: presence of the supraorbital crest of squamosal (character 20-1); squamosal otic ramus width less than half frontoparietal width at the level of crista parotica (character 24-0); and otic plate extending beyond the occipital condyles (character 25-2). The *C. aurita* group is composed of *C. joazeirensis*, *C. ornata*, *C. cranwelli*, *C. ensenadensis*, *C. ameghinorum*, *C. sagani*, *C. aurita* NHMUK PV OR18895/6, and *C. aurita*, and is supported by: squamosal and maxilla wide (character 17-1). The only unresolved node within the *C. aurita* group includes *C. sagani*, *C. aurita* NHMUK PV OR18895/6 and *C. aurita*.

Character optimization: ploidy and dorsal shield

Our optimization suggests that octaploidy was acquired in the *C. aurita* group, except by *C. cranwelli* (Fig. 2A). Although this approach does not estimate tip states, octaploidy may have occurred in four fossil representatives (*C. ameghinorum*, *C. ensenadensis*, *C. sagani* and *C. aurita* NHMUK PV OR18895/6), while the ancestor of the *Ceratophrys* crown group was diploid. *Ceratophrys rusconii* is the only known extinct species in the genus supposedly not affected by this chromosomal process, as it lies outside of the *C. aurita* group. Since octaploidy was present in the ancestor of the *C. aurita* group, the emergence of 8n (2n = 8x) species seems to have

occurred directly from a 2n ancestor, in our optimization. Despite these findings, it is important to highlight the absence of tetraploidy among terminals, which makes it impossible to obtain 4n states by this approach. However, these results suggest that genome quadruplication may have played an important role in diversification in the *Ceratophrys aurita* group. Furthermore, we recovered the ancestral state of the extinct and extant species of *Lepidobatrachus* as diploid.

Another emblematic character in Ceratophryidae is the presence of a dorsal shield. It would have evolved twice in ceratophryids according to our optimization results: in the ancestor of the *C. aurita* group and in the ancestor of *Lepidobatrachus* (Fig. 2B). This optimization was more probable than an origin at the most recent common ancestor of clade *Ceratophrys* plus *Lepidobatrachus* followed by a reversion in the *C. cornuta* group. The dorsal shield may have been present in four fossil specimens (indeed, recorded in *C. ensenadensis* and *C. ameghinorum*, and expected in *C. sagani* and *C. aurita* NHMUK PV OR18895/6). Still, the dorsal shield was expected to be absent in the ancestor of the crown group of *Ceratophrys*. The only species of *Lepidobatrachus* without a dorsal shield is *L. laevis*, but the polytomy obtained hampers discussions on the origin of the character in the group.

Discussion

We present a phylogenetic hypothesis for the family Ceratophryidae, including all extant (except for *C. testudo*) and extinct (except for *C. prisca*) species, plus two fossil representatives of extant species (i.e. *C. aurita* and *C. ornata*). Ceratophryidae was recovered as a monophyletic group, and the relationships within the family (Fig. 1) are similar to those obtained by J. D. Lynch (1982), Wild (1997), Fabrezi & Quinzio (2008) and Gómez & Turazzini (2021) based on morphological datasets, and by Faivovich *et al.* (2014) based on molecular markers. Below, we discuss the different phylogenetic positioning of species among those analyses. The sister relationship we recovered between Ceratophryidae and other anurans is novel both relating to the fossil species *Baurubatrachus pricei* and *Beelzebufo ampinga*, and if considering only extant taxa, to include a clade formed by *Telmatobufo* (Calyptocephalellidae), *Telmatobiidae*, *Hemiphraactidae*, *Alsodidae*, *Hylidae*, *Hylodidae* and *Cycloramphidae*.

Faivovich *et al.* (2014) recovered *Chacophrys* as the sister clade to *Lepidobatrachus* using direct optimization, and *Chacophrys* as the sister clade to *Ceratophrys* plus *Lepidobatrachus* by performing static parsimony

analysis. Our topology is congruent with the ones obtained by Faivovich *et al.* (2014) with static parsimony analysis, i.e. recovering *Chacophrys* as the sister clade to *Ceratophrys* plus *Lepidobatrachus*. Unfortunately, most recent analyses resulting from molecular datasets include too few representatives of the family to allow a discussion of internal relationships (Feng *et al.* 2017; Streicher *et al.* 2018; Jetz & Pyron 2018). Hime *et al.* (2020) included one representative of each genus of Ceratophryidae and obtained a result different from ours, and similar to the direct optimization of Faivovich *et al.* (2014): *Ceratophrys* sister to the clade *Chacophrys* plus *Lepidobatrachus*. The strict consensus obtained by Gómez & Turazzini (2021) is not resolved at the node of Ceratophryidae, but if one considers their majority rule consensus from an unconstrained analysis under equal weight, the relationship recovered is the same as ours: *Chacophrys* as sister to *Ceratophrys* plus *Lepidobatrachus*. The phylogenetic relationships among the genera of Ceratophryidae remain inconclusive, but the clade *Ceratophrys* plus *Lepidobatrachus* is recovered more frequently in analyses with morphological characters, and the clade *Chacophrys* plus *Lepidobatrachus* is recurrently recovered in molecular-based ones.

Gómez & Turazzini (2021)'s analysis is the most similar to ours in both scope and sample. The results we obtained are about the same regarding more inclusive groups, as Ceratophryidae was recovered as monophyletic, the relationships among genera are the same, and the groups *C. aurita* and *C. cornuta* are present. The phylogenetic relationships within *Ceratophrys*, especially concerning the fossil representatives, are very incongruent, however. Most fossil terminals of Gómez & Turazzini (2021) were recovered within the *C. ornata* group or related to this group. Most fossil terminals in our analysis, in contrast, were recovered within the *C. aurita* group. Those authors synonymized extinct species (*C. ensenadensis* and *C. rusconii*) as *C. ornata*, and recovered *C. joazeirensis* in a polytomy with all *Ceratophrys* (except *Ceratophrys* sp. MNHN UN). In comparison, we present *C. rusconii* as sister to all *Ceratophrys*, *Ceratophrys* sp. MNHN UN in a polytomy with *C. aurita* and *C. cornuta* group, and *C. ensenadensis* as a distinct species related to the *C. aurita* group.

Taxonomic accounts

***Chacophrys*.** *Chacophrys* is a monotypic genus. The status of *Ch. pierottii* was doubted by J. D. Lynch (1982) but corroborated by Maxson & Ruibal (1988) based on immunological data, and further on the description of its tadpole and ontogeny (Wild 1999). Gómez & Turazzini (2021) recovered eight autapomorphies for *Chacophrys*

in the majority rule consensus, but not in the strict consensus tree. One autapomorphy is related to skull elements and all others concern postcranial elements. We present six unequivocal autapomorphies (with four characters concerning skull elements) supporting *Chacophrys* as a distinct genus.

Lepidobatrachus. Our analysis recovers *Lepidobatrachus* as monophyletic. However, the internal relationships of the genus were not resolved and the propositions present in Barrio (1968)'s revision of the genus remains valid. Peri (1994) and Vieira (2012) proposed *Lepidobatrachus laevis* as sister clade to *L. asper* plus *L. llanensis*. In previous works, *Lepidobatrachus asper* was recovered as the sister clade to *L. laevis* plus *L. llanensis* (Faivovich *et al.* 2014; Brusquetti *et al.* 2018; Gómez & Turazzini 2021). Gómez & Turazzini (2021) did not recover unambiguous synapomorphies for *Lepidobatrachus*, but presented an osteological diagnosis for the genus based on more than 50 characters. Here, we propose nine unambiguous supporting synapomorphies for the genus (seven of them new).

Lepidobatrachus australis is an extinct species, known exclusively from fossils. It was first mentioned by Tomassini *et al.* (2011), who detailed the morphology of the specimen and assigned it to *L. laevis*. Later, this specimen was revised and described by Nicoli (2015) who raised it to species status. Gómez & Turazzini (2021) allocated this extinct species within the total group of *Lepidobatrachus*. We recognize all the autapomorphies discussed by Nicoli (2015), and we corroborate its phylogenetic allocation and its status as an extinct species by the addition of two autapomorphies: nasal partially covered by exostosis (character 2-1), and post-temporal fenestra absent (character 66-0).

Ceratophrys. Our analysis presents *Ceratophrys* including four extinct species known exclusively from fossils, and two fossil representatives of extant species. The genus is supported by six synapomorphies. We recovered the species groups proposed by J. D. Lynch (1982). In our topology, the *C. cornuta* group is formed by *C. calcarata*, *C. cornuta* and *C. stolzmanni*. The *C. aurita* group is formed by *C. ameghinorum*, *C. aurita*, *C. aurita* NHMUK PV OR18895/6, *C. cranwelli*, *C. ensenadensis*, *C. joazeirensis*, *C. ornata* and *Ceratophrys sagani*. The relationships obtained within the *C. aurita* group are unique compared to others available in the literature (i.e. J. D. Lynch 1982; Peri 1994; Wild 1997; Vieira 2012; Faivovich *et al.* 2014; Gómez & Turazzini 2021). However, they agree with previous analyses (i.e. J. D. Lynch 1982; Peri 1994) in the closer relationship between *C. aurita* and *C. ornata* compared to *C. cranwelli*.

Ceratophrys ameghinorum was recovered as part of the *C. aurita* group. The specimen was first described by Rovereto (1914) and assigned to *C. prisca*. Rovereto (1914) classified four additional specimens as *C. prisca* var. *subcornuta*, *C. prisca* var. *intermedia*, *C. prisca* var. *gigantea* and *C. prisca* plesiotype. These specimens classified by Rovereto (1914) were re-assessed by Fericola (2001) and described as a new species known only from fossils, *C. ameghinorum*, in honor of Carlos and Florentino Ameghino. We recognize all of Fericola's (2001) autapomorphies. *Ceratophrys ameghinorum* was found as sister to the crown clade formed by *C. aurita*, *C. aurita* NHMUK PV OR18895/6 and *Ceratophrys sagani* (4 Bremer; 79% jackknife), with five supporting synapomorphies: the presence of a lateral crest on squamosal and maxilla (character 18-1); presence of an oblique crest on the lateral margin of the orbit (character 19-1); presence of a supraorbital crest of the squamosal (character 20-1); otic plate of squamosal extends beyond the occipital condyles (character 25-2); and presence of the lateral plate of the squamosal (character 26-1). Gómez & Turazzini (2021) recovered a partially congruent relationship between *C. ameghinorum* and *C. aurita*. Nonetheless, given the polytomic nature of Gómez & Turazzini (2021)'s results, a strict comparison is not possible.

Ceratophrys cornuta (Linnaeus, 1758) was the first species recognized in the genus, followed by *C. aurita* (Raddi, 1823), a new species presenting a dorsal shield, and *C. megastoma* (Spix, 1824) in which the dorsal shield is absent. Günther (1858) described the morphology of *C. cornuta*, and commented that *C. cornuta* specimens present a bony dorsal shield, while *C. megastoma* does not. He also stated that *C. megastoma* does not possess an interorbital crest. The descriptions suggest that Günther (1858) misidentified *C. aurita* as *C. cornuta*, and that *C. megastoma* is a synonym of *C. cornuta*. Günther (1859) described two fossil specimens of *Ceratophrys cornuta* (NHMUK PV OR18895/6) from Lagoa Santa, Minas Gerais, Brazil, but once more the characters described match those of *C. aurita*. Báez & Gasparini (1977) referred to *C. cornuta* fossil specimens from Lagoa Santa of Günther (1859) as *C. aurita*, without explanation. Faivovich *et al.* (2014) and Nicoli (2019) commented on the taxonomic rectification of *C. cornuta* NHMUK PV OR18895/6, addressing these fossils as closely related to the *C. aurita*–*C. joazeirensis* clade. Barcelos *et al.* (2020) proposed that these specimens are fossil representatives of *C. aurita*, and this status was later corroborated (Gómez & Turazzini 2021). We recovered the fossil *C. aurita* NHMUK PV OR18895/6 nested within the *C. aurita* clade, bearing diagnostic characters of *C. aurita*, and no autapomorphies. Thus, the fossil specimens could not be

distinguished from *C. aurita*, as they share the same characters as *C. aurita* extant species.

Ceratophrys sagani was described in Barcelos *et al.* (2020) and hypothesized as closely related to the *C. aurita*–*C. joazeirensis* clade. We recovered *C. sagani* in a polytomy with *C. aurita* and *C. aurita* NHMUK PV OR18895/6, supported by the presence of: anterior portion of the nasals widened (character 7-1); squamosal with marked depressions between crests (character 21-1); and oblique crest on the lateral margin of the orbit, and the oblong-shaped choanae (character 72-1). We corroborate the species status of the extinct *C. sagani*, based on the following autapomorphies: concave epiotic eminences in the posterior view of the otoccipital (character 50-1); and vomer and sphenethmoid suture fenestrated (character 74-2).

Ceratophrys ensenadensis was described by Rusconi (1932) using four fossil specimens: three from the Frederico Hennig private collection (i.e. no. 480, no. 481, and one specimen not numbered), and one specimen from Museo de Ciencias Naturales de La Plata, Argentina, not numbered and identified as ‘*Ceratophrys ? ornata*’ at the time. The species was synonymized to *C. ornata* (Báez & Gasparini 1977). The holotype (no. 480) was proposed by Sanchíz (1998) and Nicoli (2014, 2019) as *species inquirenda* (species of doubtful identity). We searched for one specimen in the Museo de La Plata unsuccessfully. Luckily, specimens no. 480 (currently PVL 699-skull) and no. 481 (currently PVL 767-vertebral column) were discovered in the collection of the Museo del Instituto Miguel Lillo, Tucumán, Argentina. *Ceratophrys ensenadensis* was proposed as *Ceratophrys* sp. by Nicoli (2019), and later synonymized with *C. ornata* (Gómez & Turazzini 2021). Our analysis presents *C. ensenadensis* as sister to *C. ameghinorum* plus *C. aurita*, *C. sagani* and *C. aurita* NHMUK PV OR18895/6. *Ceratophrys ensenadensis* is considered here a valid species, based on the presence of one autapomorphy: vomer with one vomerine tooth (character 34-1).

We recovered *C. cranwelli* as sister to *C. ensenadensis* plus *C. ameghinorum* and the polytomy composed of *C. aurita*, *C. aurita* NHMUK PV OR18895/6 and *C. sagani*. Peri (1993a) could not find osteological differences between *C. cranwelli* and *C. ornata*, except for the apophyses of the footplate of the columella. Later topologies are also incongruent to ours: *C. cranwelli* was recovered as sister to the clade *C. aurita* plus *C. ornata* (Peri 1994), as sister to the clade *C. aurita* plus *C. calcarata* and *C. cornuta* (Wild 1997), as sister to *C. ornata* (Faivovich *et al.* 2014), and as sister to the *C. ornata* group (Gómez & Turazzini 2021).

Ceratophrys joazeirensis was presented as sister to both *C. ornata* (Mercadal 1986) and *C. aurita* (Faivovich *et al.* 2014). We recovered *C. joazeirensis* as sister to *C. ameghinorum*, *C. aurita*, *C. sagani* and *C. aurita* NHMUK PV OR18895/6. The species status of *C. joazeirensis* was doubted by Nicoli (2019), suggesting *C. aurita* and *C. joazeirensis* as synonyms, but recent works argue for its validity (e.g. Gómez & Turazzini 2021). We present four supporting autapomorphies for this species: squamosal and maxilla wide (character 17-1); vomer with thread-like postchoanal process (36-1); medial portion of postchoanal process angled posteriorly (38-2); and vomer and sphenethmoid suture forming a concavity (74-1).

The *C. cornuta* group is composed of *C. stolzmanni* as sister to the clade *C. calcarata* plus *C. cornuta* (Bremer = 3), congruent to those of Faivovich *et al.* (2014) and Gómez & Turazzini (2021), but incongruent with Peri (1994) and Vieira (2012), both of which recovered *C. stolzmanni* as sister to all *Ceratophrys* species.

Ceratophrys rusconii was described by Agnolin (2005). That author considered the species closely related to *C. ornata* and *C. cranwelli*. The specimen was assigned as *species inquirenda* (species of doubtful identity) and proposed as *Ceratophrys* sp. (Nicoli 2019). Gómez & Turazzini (2021) recovered it as a fossil representative of *C. ornata*. Our results (Fig. 1) recovered *C. rusconii* as sister to all other *Ceratophrys* species (Bremer = 5; 80% jackknife). We justify this positioning of *C. rusconii* due to the fragmentary nature of this specimen, resulting in many missing values (37% of characters scored as missing) and the presence of a set of plesiomorphic traits (e.g. absence of oblique, supra-orbital and lateral crests on the squamosal and maxilla, absence of depressions on the squamosal). There were no autapomorphies for this terminal, even though we included in our matrix the ones presented by Agnolin (2005). This result corroborates Nicoli’s (2019) suggestion that the autapomorphic traits proposed for *C. rusconii* may be variation and not sufficient to allow species determination. Therefore, the species status of *Ceratophrys rusconii* remains unclear.

Ceratophrys sp. MNHN UN from Bolivia was briefly described by Vergnaud-Grazzini (1968) and was recently proposed as *Ceratophrys* sp. (Nicoli 2019). *Ceratophrys* sp. MNHN UN was recovered as sister to all *Ceratophrys* in the analysis of Gómez & Turazzini (2021). We could not identify an unambiguous phylogenetic positioning of *Ceratophrys* sp. MNHN UN. The specimen was allocated in a polytomy with *C. aurita* and *C. cornuta* groups, in which half of the most parsimonious trees show that *Ceratophrys* sp. MNHN UN is

more closely related to the *C. aurita* group, and the other half shows that *Ceratophrys* sp. MNHN UN is more closely related to the *C. cornuta* group. A further re-assessment of *Ceratophrys* sp. MNHN UN is necessary to understand its phylogenetic position.

Casamiquela (1963) described *Wawelia gerholdi*, from the Miocene of 'Lif Mahuida', Río Negro, Argentina, as a new species of Ceratophryidae. The holotype was revised by Báez & Peri (1990) and its phylogenetic affinities were doubted. Nicoli *et al.* (2016) revised the character coding for the holotype of *Wawelia gerholdi* and changed four character states. Nicoli *et al.* (2016) considered *Wawelia* to be a junior synonym of *Calyptocephalella*, proposing it as *Calyptocephalella gerholdi* comb. nov. *Wawelia gerholdi* was recovered here as a non-ceratophryid, but we cannot discuss its position further as we recovered Calyptocephalellidae as paraphyletic. We suggest additional efforts on the systematics of *Wawelia* through a broader approach focusing on Calyptocephalellidae to address the question.

Báez & Peri (1989) described *Baurubatrachus pricei* from the Late Cretaceous of Peirópolis, Bauru Group, Minas Gerais, Brazil, as related to Ceratophryidae. The phylogenetic affinity of *Ba. pricei* was doubted and this species was recovered outside Ceratophryidae (Nicoli *et al.* 2016). Subsequently, complete preparation of the holotype revealed new information and the specimen was revised. *Baurubatrachus pricei* was recovered within Calyptocephalellidae (Báez & Gómez 2018). We present *Ba. pricei* outside Ceratophryidae, sister to *Beelzebufo ampinga*, supported by: posterior portion of otic plate of the squamosal extending beyond the level of the occipital condyles (character 25-2); and the quadrate located well posterior to the occipital condyles (character 67-3).

S. E. Evans *et al.* (2008) described the extinct species *Beelzebufo ampinga* from the Late Cretaceous of the Mahajanga Basin, Madagascar, and allocated it within Ceratophryidae, as sister to *Ceratophrys*. S. E. Evans *et al.* (2014) did a more extensive description of fragmentary elements and corroborated the phylogenetic positioning of *Beelzebufo ampinga* within Ceratophryidae. More recently, the character coding and phylogenetic positioning of *Be. ampinga* has been doubted (Ruane *et al.* 2011; Agnolin 2012; Faivovich *et al.* 2014) and it also falls within Calyptocephalellidae (Agnolin 2012). Ruane *et al.* (2011) demonstrated that the temporal likelihood of an evolutionary relationship of *Be. ampinga* with Ceratophryidae is low, and this misplaced fossil could influence divergence-time estimations in phylogenies. Báez & Gómez (2018) recovered *Beelzebufo* as a non-Ceratophryidae. We coded *Be.*

ampinga in our analysis and the species was allocated together with *Baurubatrachus* as sister to Ceratophryidae. This phylogenetic relationship is supported by the following synapomorphies: the existence of a contact between the frontoparietal and squamosal (character 15-1); width of the squamosal otic ramus narrower than half frontoparietal width at the level of the crista parotic a (character 24-1); parasphenoid alae in contact with the medial ramus of the pterygoid (character 28-1); transverse process of vertebra III much larger than the width of the corresponding neural arch (character 85-2); and transverse process of vertebra IV much larger than the width of the corresponding neural arch (character 88-2).

In our analysis, Calyptocephalellidae was recovered as paraphyletic, with *Telmatobufo* and *Wawelia* not closely related, and *Baurubatrachus* and *Beelzebufo* as sister to Ceratophryidae. By contrast, Ceratophryidae and Calyptocephalellidae were recovered as sister clades in some analyses based on morphological characters (e.g. Báez *et al.* 2009; S. E. Evans *et al.* 2014). This sister relationship was also independently recovered in analyses considering only extant species (e.g. Wiens *et al.* 2005). The sister relationship between Ceratophryidae and Calyptocephalellidae, and placement of *Baurubatrachus* and *Beelzebufo* as members of Calyptocephalellidae, are uncertain and demand efforts to include more characters and terminals in new analyses as well as better knowledge on the comparative anatomy of extant members of these families.

Comparison with Gomez & Turazzini's (2021) phylogeny

Because the Gómez & Turazzini (2021) analysis is the most recent one to include a broad sample of extant and extinct species of ceratophryids, we made careful comparisons among characters and character codifications mainly to identify where our analyses agree and to understand disagreements. The matrix of Gómez & Turazzini (2021) consists of 256 characters, but there are 36 uninformative ones, with all terminals scored as the same state. Among those that vary, we recognized duplicate characters that describe essentially the same structure, but using different nomenclature (e.g. characters 9 and 167: otic plate/lamella alaris; and characters 73 and 223: parahyoid/dorsal dermal ossification). We disagree with other character statements that mix neomorphic and transformational features (e.g. characters 114 and 149), and with the coding of others (e.g. characters 26, 73, 80, 99, 143). Furthermore, we also had difficulty recognizing some characters not accompanied by comments or illustrations (e.g. characters 36 and 158). Some incongruences between our results may be a

consequence of these differences in coding decisions. Both our study and that of Gómez & Turazzini (2021) present leading phylogenies to propose explanatory hypotheses for the evolution of ceratophryids including fossils. Despite our efforts, we perceive that the ontogeny and osteology of some species (e.g. *C. joazeirensis*, *C. stolzmanni* and *C. testudo*) remain remarkably poorly known. This certainly influences the character proposition and coding decisions in analyses and should be addressed in future studies.

Morphological homoplasies

One of the most conspicuous characters in the ceratophryid skull is the differing degree of hyperossification. Sets of cranial bones are known to exhibit modular evolution (Bardua *et al.* 2020) and the expression of hyperossification leads to severe modifications in the skull modules, such as bony articulation between the squamosal and maxilla, and a suspensorium with a posteriorly shifted jaw joint (Fabrezi *et al.* 2016; Paluh *et al.* 2020). These characteristics substantially increase the angle of the mouth opening and the bite force, making frogs with hyperossified skulls capable of feeding on large vertebrate prey (Trueb 1973; Lappin *et al.* 2017; Fabrezi *et al.* 2019; Paluh *et al.* 2020). It is common for phylogenetic analyses that include characters related to hyperossification to present homoplasies and result in artificial groupings (Báez & Gómez 2018; Paluh *et al.* 2020). With this in mind, we constructed the character statements considering all of the knowledge gathered for the relationship between homologous elements related to hyperossification, and focused on characters and character states approaching only phylogenetic-level variation. We prevented the influence of tokogenetic and ontogenetic variations using information on ceratophryid ontogeny and osteology (e.g. Peri 1994; Wild 1997; Fabrezi 2006), and raising the number of specimens analysed per terminal. Hyperossification influences directly the coding of 18 characters in our phylogeny (i.e. characters 1–4, 10, 17–22, 39, 41, 56, 65, 67, 69 and 70). Polymorphic characters were coded as missing.

The expression of a hyperossified phenotype occurs independently more than 25 times in extant anuran lineages, being recorded in Pelobatidae and Pipidae (three times), Ranoidea (eight times), Hyloidea (18 times) and Calyptocephalellidae (once) (Paluh *et al.* 2020). *Baurubatrachus pricei*, *Beelzebufo ampinga* and *Wawelia gerholdi* are hyperossified extinct species, all formerly proposed as related to Ceratophryidae. Phylogenies with broader scopes allowed the revision of those fossils, presenting them as related to other hyperossified taxa (e.g. Nicoli *et al.* 2016; Báez & Gómez 2018).

Trueb (1973) studied hyperossification in frogs and recognized three major dermostosis patterns in Anura, i.e. exostosis, co-ossification and casquing. Paluh *et al.* (2020) reconstructed the evolutionary history of cranial hyperossification in Anura and tested the influence of behaviour and microhabitat in the expression of hyperossification. Ceratophryid characters influenced by hyperossification were approached in detail through an ontogenetic perspective (e.g. Wild 1997). The non-pedicellated fang-like teeth phenotype was generated by two independent ontogenetic trajectories, i.e. paedomorphic and peramorphic (producing hyperossification) developmental patterns (Fabrezi 2001). Furthermore, the interpretation of the enlargement of the jaws in Ceratophryidae was interpreted to be caused by peramorphosis/hyperossification (Fabrezi 2006; Paluh *et al.* 2020). Exostosis occurs widely in *Ceratophrys*, and our topology suggests that an extreme phenotype evolved in two independent lineages (crown clade of *C. aurita* group, and *C. cornuta* group). Representatives of those two clades that currently occupy humid environments (e.g. *C. aurita* and *C. cornuta*) exhibit a set of crests on the maxilla and squamosal. It is not possible to discuss whether those crests were inherited from an ancestor that occupied similar environments or a semi-arid palaeoenvironment. Additionally, those crests could be maintained by other pressures as well (e.g. variation in the type of prey, phragmotic behaviour, evapotranspiration, or temperature control).

In *Ceratophrys*, the degree of hyperossification is so advanced that the sutures between bones are obliterated, forming bony ankyloses, and resulting in a single akinetic skull unit (Peri 1994; Wild 1997). This ankylosis partially influences the recognition and coding of some character states (e.g. character 10, shape of the anterior edge of the frontoparietal). However, the use of μ CT scanning enables better visualization of the bone suture morphology in some specimens (see comment in Supplemental Material 1). The dermal ornamentation pattern on the surfaces of the skull bones was tentatively used in comparative studies (e.g. Rusconi 1932; Scanferla & Agnolin 2015) and in a phylogeny (Gómez & Turazzini 2021, character 143) to infer the relationship of fossil species with extant species of Ceratophryidae. Wild (1997) observed that the pitted ornamentation in *C. cranwelli* and *C. ornata* does not vary with size (age). However, that characteristic was observed to be tokogenetically and ontogenetically variable in *C. aurita* and *Lepidobatrachus* (Peri 1994; Wild 1997). The potential phylogenetic information of this character has been doubted (Nicoli 2017, 2019).

Finally, the phylogenetic analysis of J. D. Lynch (1982) presented the otic plate shape as a character that

differentiates the species of the *C. aurita* group (sub-quadrate) from those of the *C. cornuta* group (lanceolate), but its convergent nature was never discussed. Our topology indicates that otic plate shape has evolved two times independently. The sub-quadrate otic plate convergently occurs in *C. stolzmanni* (*C. cornuta* group), and the lanceolate otic plate convergently occurs in *C. ameghinorum* (*C. aurita* group), independently. However, close observation reveals that the lanceolate otic plate in *C. ameghinorum* is wide and angled dorsally, whereas in species of the *C. cornuta* group (i.e. *C. cornuta* and *C. calcarata*) it is acute and laterally directed. Additionally, the sub-quadrate otic plate in *C. stolzmanni* is more acute, while in species of the *C. aurita* group the sub-quadrate otic plate is wide. Our topology implies that the sub-quadrate otic plate is the plesiomorphic character state and a reversion occurred in *C. stolzmanni*.

Character optimization: ploidy and dorsal shield

Polyploidy can affect evolutionary patterns in a complex way, through the emergence of new interaction pathways for gene expression as a modification from an ancestral pattern (Wertheim *et al.* 2013). Furthermore, genetic variability generated by polyploidy could increase abiotic stress tolerance (Novikova *et al.* 2020). The occurrence of polyploidy among amphibians has been associated with the temperature stress to which zygotes are potentially exposed (Mable *et al.* 2011). Once polyploidy emerged, divergent processes may lead at least one of the original genes to perform slightly different functions (M. Lynch 2004), affecting both protein sequences and their regulatory control. Due to this variability, genome duplications can substantially amplify the genetic variation of a species. Our results suggest that octaploidy arose in the most speciose and widely distributed clade of Ceratophryidae, the *C. aurita* group (see Barcelos *et al.* 2020 for a map with the species distribution). However, more detailed studies are necessary to understand the particular case of ceratophryid frogs.

Ceratophrys aurita, *C. joazeirensis* and *C. ornata*, of the *C. aurita* group, are known to be octaploid (Beçak *et al.* 1967; Bogart 1967; Schmid *et al.* 1985; Soares-Scott *et al.* 1988; Vieira *et al.* 2006). All other ceratophryids are known or inferred to be diploids (Bogart 1967; Morescalchi 1967; Barrio & Rinaldi de Chieri 1970; Mercadal 1981). Our topology suggests that the paradigm of diploid-octaploid sister species for *C. cranwelli*–*C. ornata* (J. D. Lynch 1982; Faivovich *et al.* 2014) is not recovered when the extinct species are put together in the analysis. Nonetheless, the reversion from a polyploid to a diploid state in *C. cranwelli*, as

suggested by Faivovich *et al.* (2014), is not recovered here. Our results indicate a transition from a diploid to an octaploid phenotype, when *C. ornata* is closely related to the remaining species of the *C. aurita* group (Fig. 2).

Reumer & Thiebaud (1987) developed a technique to infer the ploidy level based on the measurement of osteocyte lacunae, which was later used to infer the ploidy of fossil specimens related to *Ceratophrys* (Mercadal de Barrio & Barrio 2002). However, Mercadal de Barrio & Barrio (2002) assigned the taxonomic identity of several fossil specimens without explanation. They proposed that the MACN 14325 fossil specimen presented $4n$ ($2n = 4x$) polyploidy and thus considered it to represent *Ceratophrys prisca* var. *subcornuta*, whereas MACN 14322 was interpreted as diploid and assigned to *Ceratophrys* sp. However, these two fossil fragments, MACN 14322 (maxilla, squamosal and quadrate fragments) and MACN 14325 (other skull bones), in fact represent a single restored skull. Because the species represented by those glued fragments is uncertain, we excluded the inferred ploidy from Mercadal de Barrio & Barrio (2002) from our discussion. Despite the challenges of testing the origin and frequency of polyploidization events along the *Ceratophrys* lineage (Vieira *et al.* 2006; B. J. Evans *et al.* 2012), we suggest that there was a complex evolutionary history of polyploidization in *Ceratophrys*, encompassing unknown diploid extinct species that contributed to the genomes of extant octaploid species (i.e. *C. aurita*, *C. joazeirensis* and *C. ornata*). Furthermore, the possibility of a missing tetraploid species closely related to the *C. aurita* group is not ruled out, as the expected transition to a genome duplication would be diploid to a tetraploid, and then to an octaploid. The origin of polyploidy in Ceratophryidae, through allopolyploidy, autopolyploidy or both, remains undetermined (Schmid *et al.* 2015). Cytogenetic studies encompassing the inclusion of several specimens of diploid and octaploid species of *Ceratophrys* are needed to further understand the polyploidization process in this group of frogs (Vieira *et al.* 2006).

The dorsal shields of ceratophryids are formed by bony plates resting on the neural spines of the presacral vertebrae (Fabrezi 2006). The dorsal shield is present in extant species of the *C. aurita* group (i.e. *C. aurita*, *C. cranwelli*, *C. joazeirensis* and *C. ornata*), *L. asper* and *L. llanensis*, and is absent in *Chacophrys pierottii* and *L. laevis*, and in the *C. cornuta* group (Peri 1994; Wild 1997). Dorsal shields in *L. asper* and *L. llanensis* are formed by one or two plates in the vertebral column, that are located in a median position (Peri 1994), and differentiate at premetamorphic larval stages – 45 and

37, respectively (Quinzio & Fabrezi 2012). In the *C. aurita* group, there are more than three plates located in the vertebral column medially, but also extending laterally, and data from *C. cranwelli* indicate the number of plates can vary with age (Peri 1994; Wild 1997; Quinzio & Fabrezi 2012). In the *C. aurita* group, differentiation occurs after metamorphosis (Quinzio & Fabrezi 2012). In *C. aurita*, these plates are ankylosed to the neural spines of vertebrae, whereas in other species, the plates are attached to the vertebrae by ligaments (Peri 1994; Wild 1997; Fabrezi 2006). The differences observed in the dorsal shield morphology and formation are congruent with the results of our character optimization, suggesting this character arose twice, independently, in *Lepidobatrachus* and the *C. aurita* group. It is not clear what environmental pressures led to the expression and fixation of this homoplastic characteristic in extinct (e.g. *C. ameghinorum* and *C. ensenadensis*) and extant species of *Ceratophrys* and *Lepidobatrachus*. Our results on ploidy and dorsal shield optimization, and the placement of new fossils within the *C. aurita* group, point to a complex evolutionary history. Future analyses on ancestral state reconstructions based on trees with branch length information may shed light on this issue.

The age of the ceratophryid fossils and divergence-time estimates

Divergence-time analyses based on genetic data are severely influenced by fossils as calibration points (Near *et al.* 2005). The erroneous phylogenetic position of a fossil or the assignation of a misleading geological age of fossil-bearing rocks can lead to inaccurate divergence time estimates (Lee 1999; Benton & Ayala 2003; Conroy & van Tuinen 2003). Also, the inclusion of fossils in phylogenetic analyses has been strongly recommended to allow an accurate inference of the relationship of those fossils with other taxa and prevent misleading calibration points (Parham *et al.* 2012; Sterli *et al.* 2013). *Beelzebufo ampinga* and *Baurubatrachus pricei* were misguidedly used as calibration points for Ceratophryidae, and the in-depth study made by Báez & Gómez (2018) revealed that these fossils are not in fact related to Ceratophryidae. The time of origin and diversification of Ceratophryidae (both the stem and crown groups) are still under debate. There are hypotheses for the origin of the crown group during the early Miocene (Roelants *et al.* 2007; Wiens 2007; Pyron 2014; Feng *et al.* 2017; Hutter *et al.* 2017), middle Miocene (Ruane *et al.* 2011; Frazão *et al.* 2015; Hime *et al.* 2020), late Oligocene (Roelants *et al.* 2011; Brusquetti *et al.* 2018), late Eocene (Heinicke *et al.* 2009) and early Eocene (Jetz & Pyron 2018).

The availability of multiple fossils as calibration points provides variance in the age estimate for a given clade and generates confidence intervals on molecular age estimates (Smith & Peterson 2002; Pardo *et al.* 2020). The only fossil of the crown group of Ceratophryidae recently used as a calibration point was *Lepidobatrachus australis* for *Lepidobatrachus* (Brusquetti *et al.* 2018). Here, we placed five fossil specimens in ceratophryid phylogeny and, based on current knowledge (Fericola 2001; Nicoli 2015; Nicoli *et al.* 2017; Gómez & Turazzini 2021), present calibrations for three nodes of the family. *Ceratophrys* sp. MD-CH-06-165 (late Miocene 9.02–8.7 Ma; Zárate *et al.* 2007) is known as the most ancient record of Ceratophryidae and *Ceratophrys*. It was included in a phylogenetic analysis (Gómez & Turazzini 2021) and recovered in a polytomy within *Ceratophrys*. *Lepidobatrachus australis* (late Miocene–early Pliocene, ~7.25–3.6 Ma; Cione *et al.* 2007) is the most ancient representative of *Lepidobatrachus*, indicating that *Ceratophrys* and *Lepidobatrachus* were already well differentiated from each other during the late Miocene. *Lepidobatrachus australis* MMH 85-12-2a is recovered within *Lepidobatrachus* in our analysis; this allocation is congruent with comparative studies (Tomassini *et al.* 2011; Nicoli 2015) and the phylogeny of Gómez & Turazzini (2021). Following this reasoning, we could conjecture that the origin of Ceratophryidae may be older than expected, as *Chacophrys* is the earliest lineage that diverged in the family. However, the absence of a fossil representative of *Chacophrys* prevents further discussion of this topic. *Ceratophrys ameghinorum* MACN 14318 is the earliest record of a representative of the *C. aurita* group. The presence of a highly differentiated species such as *C. ameghinorum* in sediments of the upper Miocene–lower Pliocene (~6.8–4 Ma; Cione *et al.* 2007) suggests the split between the *C. aurita* and *C. cornuta* groups happened much earlier. The diversification pace of the ceratophryid lineage is not well established, and the inclusion of those fossils as calibration points would increase the accuracy of phylogenetic inferences and divergence-time estimates.

Conclusions

Our results support a monophyletic Ceratophryidae, with *Chacophrys* as sister to the clade *Ceratophrys* plus *Lepidobatrachus*. The relationships of Ceratophryidae to other anuran families remain unclear. The fossils once included in Ceratophryidae – *Wawelia*, *Baurubatrachus* and *Beelzebufo* – are recovered outside the family, but the last two are closely related to ceratophryids. The *C. cornuta* and *C. aurita* groups are valid; the *C. aurita* group is the most speciose, comprising most of the

fossil species described in Ceratophryidae so far. Despite the numerous studies on diverse aspects of ceratophryid evolution, there is much to be done on character variation, especially those related to hyperossification, polyploidy and polymorphisms including extant and fossil specimens.

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References

- Agnolin, F.** 2005. Un nuevo escuerzo (Anura, Leptodactylidae) del ‘Ensenadense’ Pleistoceno inferior-medio de la Provincia de Buenos Aires (Argentina), con notas sobre la clasificación del género *Ceratophrys*. *Studia Geologica Salmanticensia*, **41**, 45–55.
- Agnolin, F.** 2012. A new Calyptocephalellidae (Anura, Neobatrachia) from the Upper Cretaceous of Patagonia, Argentina, with comments on its systematic position. *Studia Geologica Salmanticensia*, **48**(2), 129–178.
- Ameghino, F.** 1899. *Sinopsis geologico-paleontologica ... En segundo censo nacional de la República Argentina, 1898, Suplemento (adiciones y correcciones), Julio de 1899*. Impf. Encuadernacion ‘La Libertad’.
- Andersson, L. G.** 1945. Batrachians from East Ecuador, collected 1937, 1938 by Wm. Clarke-Macintyre & Rolf Blomberg. *Arkiv för Zoologi*, **37A**(2), 1–88.
- Báez, A. M. & Gasparini, Z.** 1977. Orígenes y evolución de los anfibios y reptiles del Cenozoico de América del Sur. *Acta Geológica Lilloana*, **14**, 149–232.
- Báez, A. M. & Peri, S.** 1989. *Baurubatrachus pricei*, nov. gen. et sp., un anuro del Cretácico Superior de Minas Gerais, Brasil. *Anais da Academia Brasileira de Ciências*, **61**(4), 447–458.
- Báez, A. M. & Peri, S.** 1990. Revisión de *Wawelia gerholdi*, un anuro del Mioceno de Patagonia. *Ameghiniana*, **27**, 379–386.
- Báez, A. M., Moura, G. J. & Gómez, R. O.** 2009. Anurans from the Lower Cretaceous Crato Formation of northeastern Brazil: implications for the early divergence of neobatrachians. *Cretaceous Research*, **30**(4), 829–846. doi:10.1016/j.cretres.2009.01.002
- Báez, A. M. & Gómez, R. O.** 2018. Dealing with homoplasy: osteology and phylogenetic relationships of the bizarre neobatrachian frog *Baurubatrachus pricei* from the Upper Cretaceous of Brazil. *Journal of Systematic Palaeontology*, **16**(4), 279–308. doi:10.1080/14772019.2017.1287130
- Barcelos, L. A., Almeida-Silva, D., Santos, C. M. & Verdade, V. K.** 2020. Description of a new species of fossil *Ceratophrys* (Anura: Ceratophryidae) from Versalles Cave, São Paulo, Brazil. *Journal of Vertebrate Paleontology*, **40**(3), e1811293. doi:10.1080/02724634.2020.1811293
- Bardua, C., Fabre, A. C., Bon, M., Das, K., Stanley, E. L., Blackburn, D. C. & Goswami, A.** 2020. Evolutionary integration of the frog cranium. *Evolution*, **74**(6), 1200–1215. doi:10.1111/evo.13984
- Barrio, A.** 1968. Revision del genero *Lepidobatrachus* Budgett (Anura, Ceratophrynidae). *Physis*, **27**, 445–454.
- Barrio, A.** 1980. Una nueva especie de *Ceratophrys* (Anura, Ceratophryidae) del dominio Chaqueño. *Physis*, **39**(96), 21–30.
- Barrio, A. & Rinaldi de Chieri, P.** 1970. Relaciones cariosistémicas de Ceratophryidae de la Argentina (Amphibia: Anura). *Physis*, **30**, 321–329.
- Beçak, M. L., Beçak, W. & Rabello, M. N.** 1967. Further studies on polyploids amphibians (Ceratophryidae). *Chromosoma*, **22**, 192–201. doi:10.1007/BF00326728
- Bell, T.** 1843. Reptiles. Pp. 1–51 in C. Darwin (ed.) *The zoology of the voyage of the HMS Beagle, under the command of Captain Fitzroy, R.N., during the years 1832 to 1836*. Smith, Elder and Co., London.

- Benton, M. J. & Ayala, F. J.** 2003. Dating the tree of life. *Science*, **300**(5626), 1698–1700. doi:10.1126/science.1077795
- Bogart, J. P.** 1967. Chromosomes of the South American amphibian family Ceratophryidae, with a reconsideration of the taxonomic status of *Odontophrynus americanus*. *Canadian Journal of Genetics and Cytology*, **9**, 531–542. doi:10.1139/g67-057
- Boulenger, G. A.** 1890. Second report on additions to the batrachian collection in the Natural History Museum. *Proceedings of the Zoological Society of London*, **1890**, 323–328.
- Bremer, K. R.** 1994. Branch support and tree stability. *Cladistics*, **10**(3), 295–304. doi:10.1111/j.1096-0031.1994.tb00179.x
- Brusquetti, F., Netto, F., Baldo, D. & Haddad, C. F.** 2018. What happened in the South American Gran Chaco? Diversification of the endemic frog genus *Lepidobatrachus* Budgett, 1899 (Anura: Ceratophryidae). *Molecular Phylogenetics and Evolution*, **123**, 123–136. doi:10.1016/j.ympev.2018.02.010
- Budgett, J. S.** 1899. Notes on the batrachians of Paraguayan Chaco, with observations upon their breeding habits and development, especially with regard to *Phyllomedusa hypochondrialis* Cope. Also a description of a new genus. *Quarterly Journal of Microscopical Science*, **42**, 305–333.
- Caetano-Anollés, G., Nasir, A., Kim, K. M. & Caetano-Anollés, D.** 2018. Rooting phylogenies and the Tree of Life while minimizing ad hoc and auxiliary assumptions. *Evolutionary Bioinformatics*, **14**, 1–21. doi:10.1177/1176934318805101
- Casamiquela, R. M.** 1963. Sobre un par de Anuros? del Mioceno de Rio Negro (Patagonia) *Wawelia gerholdii* n. gen. et sp. (Ceratophryidae) y *Gigantobatrachus parodii* (Leptoda, Ctylidae). *Ameghiniana*, **3**(5), 141–160.
- Cione, A. L., Tonni, E. P., Bargo, S., Bond, M., Candela, A. M., Carlini, A. A., Deschamps, C. M., Dozo, M. T., Esteban, G., Goin, ... Vucetich, M. G.** 2007. Mamíferos continentales del Mioceno tardío a la actualidad en la Argentina: cincuenta años de estudios. *Ameghiniana, Publicación Especial*, **11**, 257–278.
- Conroy, C. J. & van Tuinen, M.** 2003. Extracting time from phylogenies: positive interplay between fossil and genetic data. *Journal of Mammalogy*, **84**(2), 444–455. doi:10.1644/1545-1542(2003)084<0444:ETFPPI>2.0.CO;2
- Elkan, E.** 1968. Mucopolysaccharides in the anuran defence against desiccation. *Journal of Zoology*, **155**, 19–53.
- Evans, B. J., Pyron, R. A. & Wiens, J. J.** 2012. Polyploidization and sex chromosome evolution in amphibians. Pp. 385–41 in P. Soltis & D. E. Soltis (eds) *Polyploidy and genome evolution*. Springer, Berlin & Heidelberg. doi:10.1007/978-3-642-31442-1_18
- Evans, S. E., Jones, M. E. & Krause, D. W.** 2008. A giant frog with South American affinities from the Late Cretaceous of Madagascar. *Proceedings of the National Academy of Sciences of the USA*, **105**(8), 2951–2956. doi:10.1073/pnas.0707599105
- Evans, S. E., Groenke, J. R., Jones, M. E., Turner, A. H. & Krause, D. W.** 2014. New material of *Beelzebufo*, a hyperossified frog (Amphibia: Anura) from the Late Cretaceous of Madagascar. *PLoS ONE*, **9**(1), e87236. doi:10.1371/journal.pone.0087236
- Fabrezi, M.** 2001. Variación morfológica de la dentición en anuros. *Cuadernos de Herpetología*, **15**(1), 17–28.
- Fabrezi, M.** 2006. Morphological evolution of Ceratophryinae (Anura, Neobatrachia). *Journal of Zoological Systematics and Evolutionary Research*, **44**(2), 153–166. doi:10.1111/j.1439-0469.2005.00349.x
- Fabrezi, M. & Quinzio, S. I.** 2008. Morphological evolution in Ceratophryinae frogs (Anura, Neobatrachia): the effects of heterochronic changes during larval development and metamorphosis. *Zoological Journal of the Linnean Society*, **154**(4), 752–780. doi:10.1111/j.1096-3642.2008.00420.x
- Fabrezi, M., Quinzio, S. I., Goldberg, J., Cruz, J. C., Pereyra, M. C. & Wassersug, R. J.** 2016. Developmental changes and novelties in ceratophryid frogs. *EvoDevo*, **7**, 5. doi:10.1186/s13227-016-0043-9
- Fabrezi, M., Quinzio, S. I., Goldberg, J., Cruz, J. C., Pereyra, M. C. & Wassersug, R. J.** 2019. Phenotypic variation through ontogeny: ceratophryid frogs as a model. *eLS*, 1–8. doi:10.1002/9780470015902.a0028510
- Faivovich, J., Nicoli, L., Blotto, B. L., Pereyra, M. O., Baldo, D., Barrionuevo, J. S., Fabrezi, M., Wild, E. R. & Haddad, C. F. B.** 2014. Big, bad, and beautiful: phylogenetic relationships of the horned frogs (Anura: Ceratophryidae). *South American Journal of Herpetology*, **9**(3), 207–227. doi:10.2994/SAJH-D-14-00032.1
- Farris, J. S.** 1989. The retention index and the rescaled consistency index. *Cladistics*, **5**(4), 417–419. doi:10.1111/j.1096-0031.1989.tb00573.x
- Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D. & Kluge, A. G.** 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, **12**(2), 99–124. doi:10.1111/j.1096-0031.1996.tb00196.x
- Fedorov, A., Beichel, R., Kalpathy-Cramer, J., Finet, J., Fillion-Robin, J. C., Pujol, S., ... & Buatti, J.** 2012. 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magnetic Resonance Imaging*, **30**(9), 1323–1341. doi:10.1016/j.mri.2012.05.001
- Feng, Y. J., Blackburn, D. C., Liang, D., Hillis, D. M., Wake, D. B., Cannatella, D. C. & Zhang, P.** 2017. Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. *Proceedings of the National Academy of Sciences of the USA*, **114**(29), E5864–E5870. doi:10.1073/pnas.1704632114
- Fernicola, J. C.** 2001. Una nueva especie de *Ceratophrys* (Anura, Leptodactylidae) en el Neógeno de la provincia de Buenos Aires, Argentina. *Ameghiniana*, **38**(4), 385–391.
- Frazão, A., da Silva, H. R. & de Moraes Russo, C. A.** 2015. The Gondwana breakup and the history of the Atlantic and Indian oceans unveils two new clades for early neobatrachian diversification. *PLoS ONE*, **10**(11), e0143926. doi:10.1371/journal.pone.0143926
- Frost, D. R.** 2021. *Amphibian species of the world: an online reference*. Version 6.0. American Museum of Natural History, New York, USA. Updated at: <http://research.amnh.org/herpetology/amphibia/index.html>, accessed 2 February 2021.
- Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F., ... & Raxworthy, C. J.** 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History*, **297**, 1–291. doi:10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2
- Goloboff, P. A., Farris, J. S. & Nixon, K. C.** 2008. TNT, a free program for phylogenetic analysis. *Cladistics*, **24**(5), 774–786. doi:10.1111/j.1096-0031.2008.00217.x

- Goloboff, P. A. & Catalano, S. A.** 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics*, **32**(3), 221–238. doi:10.1111/cla.12160
- Grant, T., Frost, D. R., Caldwell, J. P., Gagliardo, R. O. N., Haddad, C. F., Kok, P. J., ... & Wheeler, W. C.** 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History*, **299**, 1–262. doi:10.1206/0003-0090(2006)299[1:PSODFA]2.0.CO;2
- Gómez, R. O.** 2016. A new pipid frog from the Upper Cretaceous of Patagonia and early evolution of crown-group Pipidae. *Cretaceous Research*, **62**, 52–64. doi:10.1016/j.cretres.2016.02.006
- Gómez, R. O. & Turazzini, G. F.** 2021. The fossil record and phylogeny of South American horned frogs (Anura, Ceratophryidae). *Journal of Systematic Palaeontology*, **19**(2), 91–130. doi:10.1080/14772019.2021.1892845
- Günther, A. C. L. G.** 1858. *Catalogue of the Batrachia Salientia in the collection of the British Museum*. Order of the Trustees. Natural History Museum (London) Publications, London, 224 pp.
- Günther, A. C. L. G.** 1859. Sexual differences found in bones of some Recent and fossil species of frogs and fishes. Pp. 377–387 in *Annals and Magazine of Natural History*. Harvard University Press, Cambridge.
- Haas, A.** 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics*, **19**(1), 23–89. doi:10.1111/j.1096-0031.2003.tb00405.x
- Heinicke, M. P., Duellman, W. E., Trueb, L., Means, D. B., MacCulloch, R. D. & Hedges, S. B.** 2009. A new frog family (Anura: Terrarana) from South America and an expanded direct-developing clade revealed by molecular phylogeny. *Zootaxa*, **2211**(1), 1–35. doi:10.11646/zootaxa.2211.1.1
- Hime, P. M., Lemmon, A. R., Lemmon, E. C. M., Prendini, E., Brown, J. M., Thomson, R. C., ... & Kortyna, M. L.** 2020. Phylogenomics reveals ancient gene tree discordance in the amphibian tree of life. *Systematic Biology*, **70**(1), 49–66. doi:10.1093/sysbio/syaa034
- Hutter, C. R., Lambert, S. M. & Wiens, J. J.** 2017. Rapid diversification and time explain amphibian richness at different scales in the Tropical Andes, Earth's most biodiverse hotspot. *The American Naturalist*, **190**(6), 828–843. doi:10.1086/694319
- Jetz, W. & Pyron, R. A.** 2018. The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution*, **2**(5), 850–858. doi:10.1038/s41559-018-0515-5
- Kluge, A. G. & Farris, J. S.** 1969. Quantitative phyletics and the evolution of anurans. *Systematic Biology*, **18**(1), 1–32. doi:10.1093/sysbio/18.1.1
- Koch, N. M., Garwood, R. J. & Parry, L. A.** 2021. Fossils improve phylogenetic analyses of morphological characters. *Proceedings of the Royal Society B*, **288**, 20210044. doi:10.1098/rspb.2021.0044
- Koch, N. M. & Parry, L. A.** 2020. Death is on our side: paleontological data drastically modify phylogenetic hypotheses. *Systematic Biology*, **69**(6), 1052–1067. doi:10.1093/sysbio/syaa023
- Lappin, A. K., Wilcox, S. C., Moriarty, D. J., Stoeppler, S. A., Evans, S. E. & Jones, M. E.** 2017. Bite force in the horned frog (*Ceratophrys cranwelli*) with implications for extinct giant frogs. *Scientific Reports*, **7**, 11963. doi:10.1038/s41598-017-11968-6
- Lee, M. S. Y.** 1999. Molecular clock calibrations and metazoan divergence dates. *Journal of Molecular Evolution*, **49**, 385–391. doi:10.1007/pl00006562
- Linnaeus, C.** 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Tomus I. Editio decima, reformata. Salvius, Holmiae, Fourth Edition, 824 pp. doi:10.5962/bhl.title.542
- Lloyd, G. T.** 2016. Estimating morphological diversity and tempo with discrete character-taxon matrices: implementation, challenges, progress, and future directions. *Biological Journal of the Linnean Society*, **118**(1), 131–151. doi:10.1111/bij.12746
- Lynch, J. D.** 1971. Evolutionary relationships, osteology, and zoogeography of leptodactyloid frogs. *University of Kansas Museum of Natural History, Miscellaneous Publications*, **53**, 1–238.
- Lynch, J. D.** 1982. Relationships of the frogs of the genus *Ceratophrys* (Leptodactylidae) and their bearing on hypotheses of Pleistocene forest refugia in South America and punctuated equilibria. *Systematic Biology*, **31**(2), 166–179. doi:10.1093/sysbio/31.2.166
- Lynch, M.** 2004. Gene duplication and evolution. Pp 33–47 in A. Moya & E. Font (eds) *Evolution: from molecules to ecosystems*. Oxford University Press, New York.
- Mable, B. K., Alexandrou, M. A. & Taylor, M. I.** 2011. Genome duplication in amphibians and fish: an extended synthesis. *Journal of Zoology*, **284**(3), 151–182. doi:10.1111/j.1469-7998.2011.00829.x
- Maddison, W. P. & Maddison, D. R.** 2018. *Mesquite: a modular system for evolutionary analysis*. Version 3.6 (build 917). Updated at: mesquiteproject.org, accessed 8 March 2020.
- Marjanović, D. & Laurin, M.** 2019. Phylogeny of Paleozoic limbed vertebrates reassessed through revision and expansion of the largest published relevant data matrix. *PeerJ*, **6**, e5565. doi:10.7717/peerj.5565
- Maxson, L. R. & Ruibal, R.** 1988. Relationships of frogs in the leptodactylid subfamily Ceratophryinae. *Journal of Herpetology*, **22**(2), 228–231. doi:10.2307/1564003
- Mercadal, I. T.** 1981. Determinación del nivel de ploidia en ejemplares preservados del género *Ceratophrys*. *Amphibia-Reptilia*, **3**(4), 205–212. doi:10.1163/156853881X00320
- Mercadal, I. T.** 1986. *Ceratophrys joazeirensis* sp. n. (Ceratophryidae, Anura) del noreste de Brasil. *Amphibia-Reptilia*, **7**(4), 313–334. doi:10.1163/156853886X00145
- Mercadal, I. T.** 1988. Sobre la validez de *Ceratophrys testudo* Andersson, 1945 (Amphibia, Ceratophryidae). *Amphibia-Reptilia*, **9**(1), 1–6. doi:10.1163/156853888X00143
- Mercadal de Barrio, I. T. & Barrio, A.** 2002. Tetraploidy in *Ceratophrys* (Anura, Leptodactylidae), analysis of the fossil record. *Iheringia. Série Zoologia*, **92**(3), 17–31. doi:10.1590/S0073-47212002000300003
- Morescalchi, A.** 1967. The close karyological affinities between *Ceratophrys* and *Pelobates* (Amphibia, Salienta). *Experientia*, **23**, 1071–1072. doi:10.1007/BF02136458
- Near, T. J., Meylan, P. A. & Shaffer, H. B.** 2005. Assessing concordance of fossil calibration points in molecular clock studies: an example using turtles. *The American Naturalist*, **165**(2), 137–146. doi:10.1086/427734

- Nicoli, L.** 2014. Reappraisal of a ceratophryid frog from the Oligocene of Patagonia: assignment to *Ceratophrys* and new insight about its provenance. *Ameghiniana*, **51**(3), 184–194. doi:10.5710/AMGH.18.02.2014.1972
- Nicoli, L.** 2015. New fossil species of the extant genus *Lepidobatrachus* (Anura, Ceratophryidae) from the Late Miocene–Early Pliocene of central Argentina. *Journal of Vertebrate Paleontology*, **35**(5), e981636. doi:10.1080/02724634.2015.981636
- Nicoli, L.** 2016. A fossil ceratophryid anuran from the late Miocene of west-central Argentina. *Salamandra*, **52**(2), 153–160.
- Nicoli, L.** 2017. The presence of *Lepidobatrachus* (Anura, Ceratophryidae) in the Neogene of the La Pampa Province, Argentina. *Ameghiniana*, **54**(6), 700–706. doi:10.5710/AMGH.08.06.2017.3064
- Nicoli, L.** 2019. The fossil record of *Ceratophrys* Wied-Neuwied (Anura: Ceratophryidae): a revision and update of fossil South American horned frogs. *Zootaxa*, **4658**(1), 37–68. doi:10.11646/zootaxa.4658.1.2
- Nicoli, L., Muzzopappa, P. & Faivovich, J.** 2016. The taxonomic placement of the Miocene Patagonian frog *Wawelia gerholdi* (Amphibia: Anura). *Alcheringa*, **40**(2), 153–160. doi:10.1080/03115518.2016.1101998
- Nicoli, L., Tomassini, R. L. & Montalvo, C. I.** 2017. The oldest record of *Ceratophrys* (Anura, Ceratophryidae) from the Late Miocene of central Argentina. *Journal of Vertebrate Paleontology*, **37**(1), e1261360. doi:10.1080/02724634.2017.1261360
- Novikova, P. Y., Brennan, I. G., Booker, W., Mahony, M., Doughty, P., Lemmon, A. R., ... & Donnellan, S. C.** 2020. Polyploidy breaks speciation barriers in Australian burrowing frogs *Neobatrachus*. *PLoS Genetics*, **16**(5), e1008769. doi:10.1371/journal.pgen.1008769
- Ohno, S.** 1970. *Evolution by gene duplication*. Springer Science & Business Media, New York, 160 pp. doi:10.1007/978-3-642-86659-3
- Paluh, D. J., Stanley, E. L. & Blackburn, D. C.** 2020. Evolution of hyperossification expands skull diversity in frogs. *Proceedings of the National Academy of Sciences of the USA*, **117**(15), 8554–8562. doi:10.1073/pnas.2000872117
- Pardiñas, U. F. J.** 2001. Condiciones áridas durante el Holoceno Temprano en el sudoeste de la provincia de Buenos Aires (Argentina): vertebrados y tafonomía. *Ameghiniana*, **38**, 227–236.
- Pardo, J. D., Lennie, K. & Anderson, J. S.** 2020. Can we reliably calibrate deep nodes in the tetrapod tree? Case studies in deep tetrapod divergences. *Frontiers in Genetics*, **11**, 1159. doi:10.3389/fgene.2020.506749
- Parham, J. F., Donoghue, P. C., Bell, C. J., Calway, T. D., Head, J. J., Holroyd, P. A., ... & Benton, M. J.** 2012. Best practices for justifying fossil calibrations. *Systematic Biology*, **61**(2), 346–359. doi:10.1093/sysbio/syr107
- Patterson, C.** 1981. Significance of fossils in determining evolutionary relationships. *Annual Review of Ecology and Systematics*, **12**(1), 195–223. doi:10.1146/annurev.es.12.110181.001211
- Peng, J.** 2019. Gene redundancy and gene compensation: an updated view. *Journal of Genetics and Genomics*, **46**(7), 329–333. doi:10.1016/j.jgg.2019.07.001
- Pérez-Ben, C. M., Turazzini, G. F. & Gómez, R. O.** 2019. A Last Glacial anuran assemblage from the inland Pampas of South America provides insights into climate and environments during Marine Isotope Stage 3. *Journal of Vertebrate Paleontology*, **39**(3), e1627365. doi:10.1080/02724634.2019.1627365
- Peri, S. I.** 1993a. *Ceratophrys* (Anura, Leptodactylidae) en el Holoceno de Laguna Los Tres Reyes, Provincia de Buenos Aires, Argentina. *Ameghiniana*, **30**(1), 3–7.
- Peri, S. I.** 1993b. La validez de *Ceratophrys testudo* Andersson, 1945 (Leptodactylidae, Ceratophryinae): aspectos morfológicos de su reconocimiento. *Alytes*, **11**(3), 107–116.
- Peri, S. I.** 1994. *Relaciones evolutivas de las especies de la subfamilia Ceratophryinae [Anura: Leptodactylidae]*. Unpublished PhD thesis, Universidad Nacional de La Plata, 223 pp.
- Prince, V. E. & Pickett, F. B.** 2002. Splitting pairs: the diverging fates of duplicated genes. *Nature Reviews Genetics*, **3**(11), 827–837. doi:10.1038/nrg928
- Pyron, R. A.** 2014. Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. *Systematic Biology*, **63**(5), 779–797. doi:10.1093/sysbio/syu042
- Pyron, R. A. & Wiens, J. J.** 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, **61**(2), 543–583. doi:10.1016/j.ympev.2011.06.012
- Quinzio, S. & Fabrezi, M.** 2012. Ontogenetic and structural variation of mineralizations and ossifications in the integument within ceratophryid frogs (Anura, Ceratophryidae). *The Anatomical Record*, **295**(12), 2089–2103. doi:10.1002/ar.22601
- R Development Core Team.** 2021. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Updated at: <https://www.r-project.org/>, accessed 10 February 2021.
- Raddi, G.** 1823. Continuazione della descrizione dei rettili Brasiliani. *Memorie Matematica et di Fisica della Società Italiana della Scienze*, **19**, 58–73.
- Reig, O. A.** 1958. Notas para la actualización del conocimiento de la fauna de la Formación Chapalmalal. II, Amphibia, Reptilia, Aves, Mammalia (Masupialis: Didelphidae: Boryaenidae). *Acta Geológica Lilloana*, **2**, 255–283.
- Reig, O. A. & Limeses, C. E.** 1963. Un nuevo género de anuros ceratofrínidos del distrito chaqueño. *Physis*, **24**, 113–128.
- Reumer, J. W. F. & Thiebaud, C. H.** 1987. Osteocyte lacunae size in the genus *Xenopus* (Pipidae). *Amphibia-Reptilia*, **8**, 315–320. doi:10.1163/156853887X00090
- Roelants, K., Gower, D. J., Wilkinson, M., Loader, S. P., Biju, S. D., Guillaume, K., ... & Bossuyt, F.** 2007. Global patterns of diversification in the history of modern amphibians. *Proceedings of the National Academy of Sciences of the USA*, **104**(3), 887–892. doi:10.1073/pnas.0608378104
- Roelants, K., Haas, A. & Bossuyt, F.** 2011. Anuran radiations and the evolution of tadpole morphospace. *Proceedings of the National Academy of Sciences of the USA*, **108**(21), 8731–8736. doi:10.1073/pnas.1100633108
- Rovereto, C.** 1914. Los estratos araucanos y sus fósiles. *Anales del Museo Nacional Historia Natural de Buenos Aires*, **25**, 1–249.
- Ruane, S., Pyron, R. A. & Burbrink, F. T.** 2011. Phylogenetic relationships of the Cretaceous frog *Beelzebubo* from Madagascar and the placement of fossil constraints based on temporal and phylogenetic evidence.

- Journal of Evolutionary Biology*, **24**(2), 274–285. doi:10.1111/j.1420-9101.2010.02164.x
- Rusconi, C.** 1932. La presencia de anfibios ('Ecaudata') y de aves fósiles en el piso ensenadense de Buenos Aires. *Anales de la Sociedad Científica Argentina*, **113**, 145–149.
- Sabbag, A. F., Lyra, M. L., Zamudio, K. R., Haddad, C. F., Feio, R. N., Leite, F. S., ... & Brasileiro, C. A.** 2018. Molecular phylogeny of Neotropical rock frogs reveals a long history of vicariant diversification in the Atlantic forest. *Molecular Phylogenetics and Evolution*, **122**, 142–156. doi:10.1016/j.ympev.2018.01.017
- Sanchiz, B.** 1998. *Salientia, Encyclopedia of Palaeoherpetology*. Fourth Edition. Verlag Dr Friedrich Pfeil, Munich, 283 pp.
- Scanferla, A. & Agnolin, F. L.** 2015. Nuevos aportes al conocimiento de la herpetofauna de la formación Cerro Azul (mioceno superior), provincia de La Pampa, Argentina. *Papéis Avulsos de Zoologia*, **55**(23), 323–333.
- Schmid, M., Haaf, T. & Schempp, W.** 1985. Chromosome banding in Amphibia. IX. The polyploid karyotypes of *Odontophrynus americanus* and *Ceratophrys ornata* (Anura, Leptodactylidae). *Chromosoma*, **91**, 172–184. doi:10.1007/BF00328215
- Schmid, M., Evans, B. J. & Bogart, J. P.** 2015. Polyploidy in Amphibia. *Cytogenetic and Genome Research*, **145**(3–4), 315–330. doi:10.1159/000431388
- Sereno, P. C.** 2007. Logical basis for morphological characters in phylogenetics. *Cladistics*, **23**(6), 565–587. doi:10.1111/j.1096-0031.2007.00161.x
- Smith, A. B. & Peterson, K. J.** 2002. Dating the time of origin of major clades. *Annual Review of Earth and Planetary Sciences*, **30**, 65–88. doi:10.1146/annurev.earth.30.091201.140057
- Soares-Scott, M. D., Trajtengertz, I., Soma, M. & Beçak, M. L.** 1988. C and Ag as bands of the octaploid untanha frog *Ceratophrys dorsata* (*C. aurita*) (8n = 104, Amphibia, Anura). *Brazilian Journal of Genetics*, **11**, 625–631.
- Spix, J. B.** 1824. *Animalia nova sive species novae testudinum et ranarum, quas in itinere per Brasiliam annis 1817–1820 jussu et auspiciis Maximiliani Josephi I. Bavariae Regis*. F. S. Hübschmann, Munich.
- Steindachner, F.** 1882. Batrachologische Beiträge. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Classe*, **85**, 188–194.
- Sterli, J., Pol, D. & Laurin, M.** 2013. Incorporating phylogenetic uncertainty on phylogeny-based palaeontological dating and the timing of turtle diversification. *Cladistics*, **29**, 233–246. doi:10.1111/j.1096-0031.2012.00425.x
- Streicher, J. W., Miller, E. C., Guerrero, P. C., Correa, C., Ortiz, J. C., Crawford, A. J., ... & Wiens, J. J.** 2018. Evaluating methods for phylogenomic analyses, and a new phylogeny for a major frog clade (Hylidae) based on 2214 loci. *Molecular Phylogenetics and Evolution*, **119**, 128–143. doi:10.1016/j.ympev.2017.10.013
- Toledo, R. C. & Jared, C.** 1993. The calcified dermal layer in anurans. *Comparative Biochemistry and Physiology – Part A*, **104**(3), 443–448.
- Tomassini, R. L., Agnolin, F. & Oliva, C.** 2011. First fossil record of the genus *Lepidobatrachus* Budgett, 1899 (Anura, Ceratophryidae), from the early Pliocene of Argentina. *Journal of Vertebrate Paleontology*, **31**(5), 1005–1009. doi:10.1080/02724634.2011.596602
- Trueb, L.** 1973. Bones, frogs, and evolution. Pp 65–132 in J. L. Vial (ed.) *Evolutionary biology of the anurans: contemporary research on major problems*. University of Missouri Press, Columbia.
- Vergnaud-Grazzini, C.** 1968. Amphibiens pléistocènes de Bolivie. *Bulletin de la Société géologique de France*, **10**, 688–695.
- Vieira, K. S.** 2012. *Estudo filogenético e biogeográfico da Subfamília Ceratophryinae (Anura-Ceratophryidae)*. Unpublished PhD thesis, Universidade Federal da Paraíba, Brazil, 470 pp.
- Vieira, K. S., Silva, A. Z. & Arzabe, C.** 2006. Cranial morphology and karyotypic analysis of *Ceratophrys joazeirensis* (Anura: Ceratophryidae, Ceratophryinae): taxonomic considerations. *Zootaxa*, **1320**(1), 57–68. doi:10.11646/zootaxa.1320.1.6
- Wertheim, B., Beukeboom, L. W. & Van de Zande, L.** 2013. Polyploidy in animals: effects of gene expression on sex determination, evolution and ecology. *Cytogenetic and Genome Research*, **140**(2–4), 256–269. doi:10.1159/000351998
- Wied-Neuwied, M. A. P.** 1822. Abbildungen zur Naturgeschichte Brasiliens. *Verlage des Grossherzoglichen Sächsischen Privat Landes-Industrie-Comptoirs*. doi:10.5962/bhl.title.51486
- Wiens, J. J.** 2007. Global patterns of species richness and diversification in amphibians. *American Naturalist*, **170**, S86–S106. doi:10.1086/519396
- Wiens, J. J., Fetzner Jr, J. W., Parkinson, C. L. & Reeder, T. W.** 2005. Hylid frog phylogeny and sampling strategies for speciose clades. *Systematic Biology*, **54**(5), 778–807. doi:10.1080/10635150500234625
- Wild, E. R.** 1997. *The ontogeny and phylogeny of ceratophryine frogs (Anura: Leptodactylidae)*. Unpublished PhD thesis, University of Kansas, Lawrence, 492 pp.
- Wild, E. R.** 1999. Description of the chondrocranium and osteogenesis of the chacoan burrowing frog, *Chacophrys pierotti* (Anura: Leptodactylidae). *Journal of Morphology*, **242**(3), 229–246. [https://doi.org/10.1002/\(SICI\)1097-4687\(199912\)242:3<229::AID-JMOR3>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1097-4687(199912)242:3<229::AID-JMOR3>3.0.CO;2-N)
- Woodhouse, M., Burkart-Waco, D. & Comai, L.** 2009. Polyploidy. *Nature Education*, **2**(1), 1.
- Zárate, M. A., Schultz, P. H., Blasi, A., Heil, C., King, J. & Hames, W.** 2007. Geology and geochronology of type Chasicuan (late Miocene) mammal-bearing deposits of Buenos Aires (Argentina). *Journal of South American Earth Sciences*, **23**(1), 81–90. doi:10.1016/j.jsames.2006.09.018
- Zhang, P., Liang, D., Mao, R. L., Hillis, D. M., Wake, D. B. & Cannatella, D. C.** 2013. Efficient sequencing of anuran mtDNAs and a mitogenomic exploration of the phylogeny and evolution of frogs. *Molecular Biology and Evolution*, **30**(8), 1899–1915. doi:10.1093/molbev/mst091

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