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Egg clutch structure of *Rhinella rumbolli* (Anura: Bufonidae), a toad from the Yungas of Argentina, with a review of the reproductive diversity in *Rhinella*

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Abstract. *Rhinella rumbolli* is a poorly known, medium-sized toad endemic to the Yungas of Argentina. Recent fieldwork allowed observing its peculiar oviposition mode, which is described in this paper. A review of literature and examination of museum material indicate that oviposition in *Rhinella* can vary from strings of eggs in a gelatinous tube (uniserial, biserial or multiserial) to open clumps. Clutch features in *Rhinella* provide informative variation for taxonomic and phylogenetic studies and help to understand the relationships in this large genus of true toads.

Key words. Argentina, *Rhinella veraguensis* group, oviposition modes, open clumps, strings, water mould.

Introduction

Rhinella is a species-rich bufonid genus, widely distributed throughout different Neotropical ecoregions (Frost 2014). Its 87 species are grouped into seven species groups: the *R. acrolopha* (formerly *Rhamphophryne*), *R. crucifer*, *R. granulosa*, *R. margaritifera*, *R. marina*, *R. spinulosa*, and *R. veraguensis* groups (Duellman & Schulte 1992, Pramuk 2006, Grant & Bolivar-G. 2014), although the monophyly of some of them has not been properly corroborated (Pramuk 2006, Chaparro et al. 2007, Pyron & Wiens 2011; Moravec et al. 2014). The reproductive biology of several species of the *R. marina*, *R. granulosa*, *R. crucifer*, *R. margaritifera*, and *R. spinulosa* groups is relatively well documented. However, this knowledge is deficient and limited merely to tadpole descriptions in some species of the *R. veraguensis* group, and to reports of number, colouration, and size of presumably mature oocytes in species of the *R. acrolopha* group (Appendix 1).

Rhinella rumbolli (Carrizo, 1992) is a medium-sized toad from the Yungas of Argentina. This species is a largely aquatic inhabitant of forest streams and rivers, and males vocalize at the stream margins where the current is slower (Carrizo 1992, Haad et al. 2014). Adults have been observed active or hiding in the leaf litter near rivers during the day (MOP pers. obs., D.E. Cardozo & J.M. Ferro pers. comms.). The tadpoles of *R. rumbolli* have features intermediate between the morphology of pond tadpoles and that of gastromyzophorous forms exclusive to the *R. veraguensis* group (Haad et al. 2014).

In this paper, we describe the clutch structure and reproductive mode of *Rhinella rumbolli*, and review the current knowledge on clutch structures and oviposition modes in *Rhinella*. In addition, we report the infection of clutches by water moulds of the family Saprolegniaceae. Finally, we interpret these findings in the context of the current phylogenetic hypotheses of *Rhinella* and discuss their systematic relevance.

Material and methods

A field survey was conducted on 25–26 August 2013, at Trigo Pampa River, Ocloyas (Jujuy, Argentina; 23°55'33.2" S, 65°15'13.1" W, datum WGS84; 1,500 m above sea level). Trigo Pampa is a typical mountain river, with bedrock and regimes of large floods during summer. The habitat corresponds to a type of rainforest known as 'cloud forest', and is included in the Yungas Ecoregion (Cabrera 1994). Numerous males of *Rhinella rumbolli* were heard calling between 20:00 and 23:30 hours, and at this time several pairs were observed in axillar amplexus in a mountain river. A few specimens of *R. arenarum* and *Hypsiboas riojanus* were also active in the area. The next morning, egg clutches were collected from the same stream and preserved in 10% formalin, and many were retained for rearing the embryos. We assigned the clutches to *R. rumbolli* by rearing some of them to tadpoles, which can be easily identified (see Haad et al. 2014), and confirmed their identity by comparison with a complete clutch (LGE 6415) that we obtained from an amplectant pair in the laboratory (LGE 6427 x LGE 6428). Eggs per clutch were counted and egg diameters measured to the nearest 0.01 mm using a Leica M205 A stereomicroscope with a DFC295 camera.

In order to explore variation among the species of *Rhinella*, published descriptions on reproductive biology (oviposition mode, number of eggs/oocytes, pigmentation, and ovum size) were compiled and are summarized in Appendix 1. This table also contains additional information about the reproductive biology of taxa with deficient data, which we obtained from photographic records, egg clutches in herpetological collections, and/or dissection of gravid females.

The terminology used to describe clutch structures is that of Salthe (1963) and Altig & McDiarmid (2007). For the composition of species groups of *Rhinella*, we follow Duellman & Schulte (1992) with some modifications based on subsequent publications (e.g., Baldissera et al. 2004, Pramuk 2006, Narvaes & Rodrigues 2009; Grant & Bolivar-G. 2014). Studied specimens are housed in the following herpetological collections: División Herpetología, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' – CONICET (MACN), Buenos Aires, Argentina; Instituto de Herpetología, Fundación Miguel Lillo (FML), Tucumán, Argentina; and Laboratorio de Genética Evolutiva (LGE), Instituto de Biología Subtropical (CONICET – Universidad Nacional de Misiones), Posadas, Misiones, Argentina. All the examined material is listed in Appendix 2.

Results

We found 25 clutches of *Rhinella rumbolli* in natural situations. All clutches had been deposited in places with shallow, slow-flowing or stagnant water, on the nude sandy bottom or among submerged vegetation of the river (mode

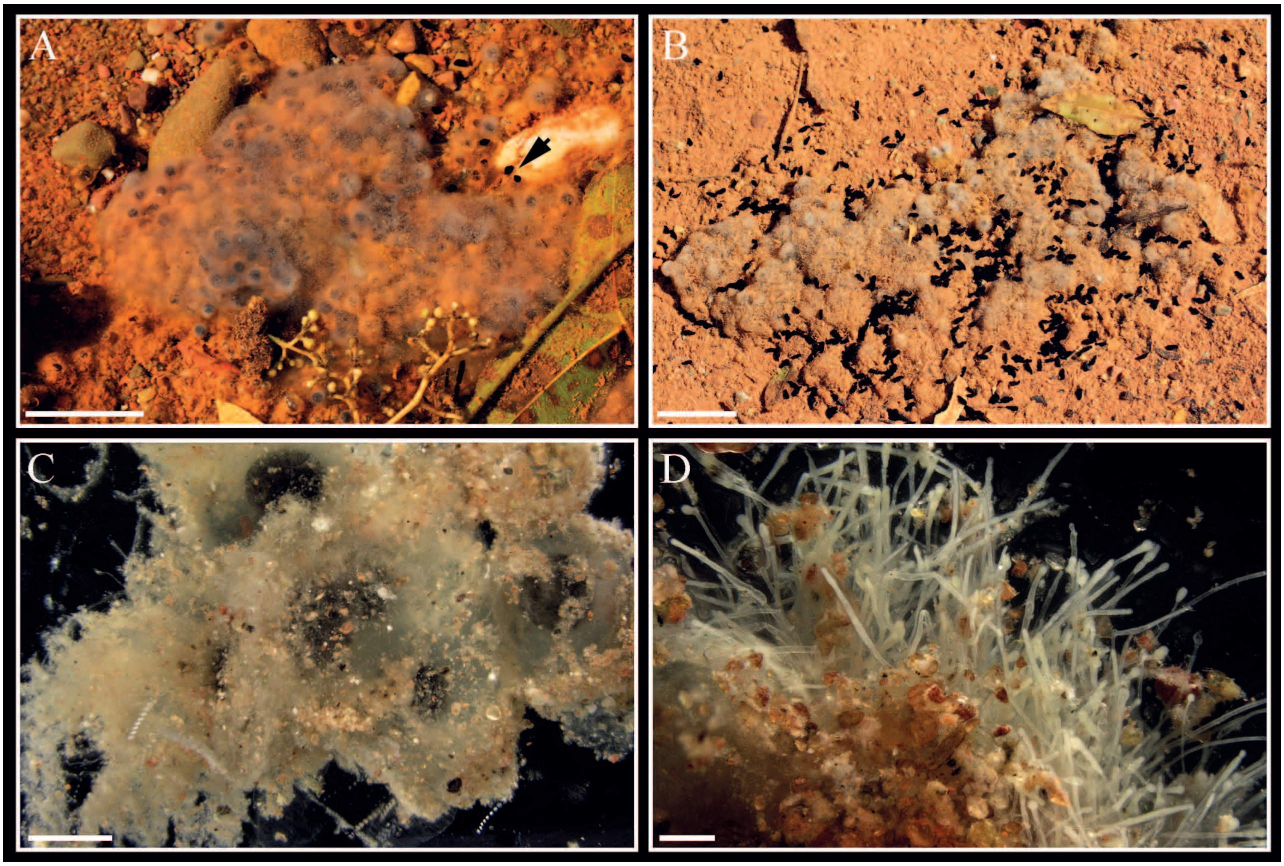

Figure 1. A, B) Clutches of *Rhinella rumbolli* photographed in situ in the Trigo Pampa river, at different stages of development (scale bars = 2 cm); the arrow points out hatched embryos at Gosner (1960) stages 17–18; C) Detail of infected eggs (scale bar = 2 mm); D) showing the reproductive structures of water mould (scale bar = 0.5 mm).

2 of Haddad & Prado 2005). Most clutches were severely infected with water mould (Oomycota: Saprolegniacea). In less severe cases, recently hatched embryos and successive developmental stages were observed near the spoiled eggs (Fig. 1).

A clutch of *Rhinella rumbolli* consists mostly of a monolayered clump of eggs and lacks a surrounding matrix. It is an "open clump" as defined by Altig & McDiarmid (2007) in that the surface of adjacent eggs is firm enough to form interstices (Fig. 2A). The only difference to Altig & McDiarmid's (2007) definition, where clumps do not have outlier eggs, is that we observed single and paired egg groups at the edges of the main group. The clutch obtained in the lab, the only one we are certain is complete, was approximately 20 cm in diameter, and consisted of 2,177 eggs. The individual eggs are relatively large (3.85 mm in average diameter) and have a thick (about 0.97 mm), sticky jelly that makes adjacent eggs adhere to each other. The ova are also relatively large (about 1.91 mm) and have a pigmented, dark animal pole and a whitish vegetal pole.

The dissection of an adult female of *Rhinella quechua*, another species of the *R. veraguensis* group, failed to produce any oocytes from the ovisac, and we only found mature ovarian oocytes. The right ovary contained 507 oocytes with a pigmented animal pole and a mean diameter of 1.27 ± 0.05 mm (range 1.19–1.37; N = 20). Egg counts and descriptions from preserved clutches of *R. achalensis*, *R. arenarum*, *R. azarai*, *R.* cf. *cerradensis*, *R. fernandezae*, *R. major*, *R. ornata*, *R. quechua*, *R. schneideri*, and *R. spinulosa* are detailed in Appendix 1.

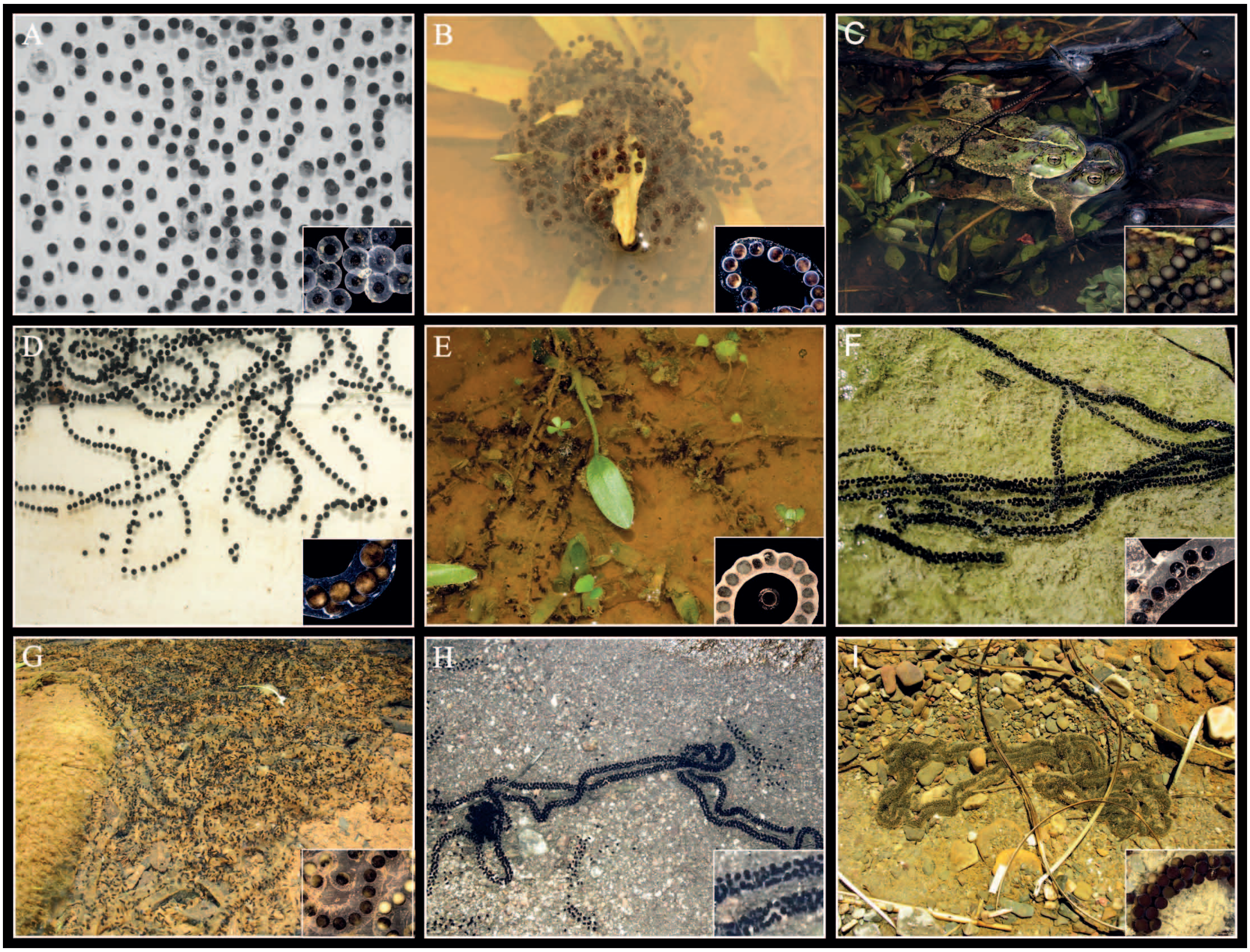

Figure 2. Clutches from nine species of the *Rhinella veraguensis* (RV), *R. granulosa* (RG), *R. marina* (RM), *R. crucifer* (RC), and *R. spinulosa* (RS) groups. The insets show details of each clutch and the arrangement of eggs. A) *R. rumbolli* RV (LGE 6415), portion of a non-infected clutch with independent eggs and interstices between them; B) *R. azarai* RG (LGE 7329); C) *R. dorbignyi* RG (unvouchered); D) *R. fernandezae* RG (LGE 7008); E) *R. major* RG (unvouchered); F) *R. arenarum* RM (unvouchered); G) *R. ornata* RC (LGE 8824); H) *R. achalensis* RS (unvouchered); I) *R. spinulosa spinulosa* RS (LGE 6795).

Discussion

Oviposition modes in *Rhinella*

Oocytes of amphibians are enclosed by a thin vitelline membrane, which is composed of glycoproteins that are synthesised and secreted during oocyte maturation in the ovary. Surrounding them are a number of jelly layers produced by oviduct secretions that are deposited around the oocytes as they flow through the different regions of the oviduct (Salthe 1963, Jego et al. 1980, Arranz et al. 1997). The plesiomorphic and most common oviposition mode reported in *Rhinella* consists of a string, composed of a uni- or bilayered jelly tube with numerous eggs. It is characteristic of the *R. crucifer*, *R. granulosa*, *R. margaritifera*, *R. marina*, and partly of the *R. spinulosa* species groups (see Appendix 1). Eggs within the strings can adopt three well-defined configurations: (i) uniserial, where the eggs are aligned inside smooth (e.g., *R. dorbignyi*, Fig. 2C) or scalloped strings (*R. major* and *R. fernandezae*, Figs. 2D–E); (ii) biserial, where the eggs are staggered inside the tube (e.g., *R. arenarum*, Fig. 2F); or (iii) multiserial, where groups of eggs are laid in a large, thick jelly tube (e.g., *R. achalensis* and *R. spinulosa*; Figs. 2H–I). We consider this latter case to be multiserial strings instead of strands (sensu Altig & McDiarmid 2007), because we found structural differences in that the eggs are ordered, and the jelly tube is definitively not flimsy or indistinct but firm and very resistant like that of regular uniserial or biserial strings. *Rhinella achalensis* has so far not been included in phylogenetic analyses, and given that some species of the *R. spinulosa* group deposit their eggs in uniserial strings, multiserial strings could represent a putative synapomorphy of at least an internal clade of this species group. In turn, the open clump of *R. rumbolli* differs remarkably from the strings with different morphologies known in *Rhinella*. Unlike other *Rhinella* eggs, there is no surrounding tube-like matrix enveloping the whole clutch, but the individual capsule of jelly layers is easily distinguished, thick and firm (Appendix 1). Detailed histochemical and molecular studies that unveil the fine structure of the oviducts and their secretions in *R. rumbolli* and other species of *Rhinella* producing strings can help to understand the formation, morphological diversity, and function of the latter.

The "*Rhinella veraguensis*" group, as currently defined, is recovered as polyphyletic in the most inclusive phylogenetic analysis of *Rhinella* (Pyron & Wiens 2011). Part of the *R. veraguensis* group (*R. chavin*, *R. manu*, and *R. nesiotes*) is sister to the *R. acrolopha* group, whereas the remaining species of the *R. veraguensis* group (*R. amboroensis* and *R. veraguensis*) are basal to the latter clade plus the *R. margaritifera* group. The biology of the species of the "*R. veraguensis*" and *R. acrolopha* groups remains poorly known, with no other reports on oviposition other than the one presented here. In several species of these groups, large unpigmented ovarian eggs have been observed (see Appendix 1). The character state unpigmented eggs optimises as a synapomorphy of the clade composed by *Rhinella chavin*, *R. nesiotes*, *R. manu* (with unknown condition), and species of the *R. acrolopha* group. *Rhinella justinianoi*, *R. multiverrucosa*, and *R. yanachaga* also have unpigmented eggs (Harvey & Smith 1994, Lehr et al. 2005, 2007) and are most likely related to this clade (for the phylogenetic relationships of *R. yanachaga* see Moravec et al. 2014). On the other hand, *R. stanlaii* and *R. sternosignata*, two species tentatively assigned to the *R. margaritifera* group, also have unpigmented eggs (Lötters & Köhler 2000, La Marca & Mijares-Urrutia 1996). Although both species were not included in a phylogenetic analysis, this condition could be homoplastic considering the position of putative related species of the *R. margaritifera* group.

Several authors have suggested on the basis of the occurrence of large unpigmented oocytes and collecting places distant from water bodies that terrestrial oviposition and an endotrophic developmental mode (direct development or nidicolous larvae) likely occur in the *Rhinella acrolopha* group and in some species of the "*R. veraguensis*" group (Duellman & Toft 1979, Thibaudeau & Altig 1999, Lehr et al. 2007, van Bocxlaer et al. 2010, Grant & Bolivar-G. 2014). A redefined *R. acrolopha* group, including the phylogenetically related species of the *R. veraguensis* group (i.e., *R. chavin*, *R. nesiotes*, and *R. yanachaga*; Pyron & Wiens 2011, Moravec et al. 2014), would comprise species with large unpigmented eggs and render the *R. veraguensis* group possibly limited to species that breed in lotic water bodies and have exotrophic tadpoles (i.e., *R. chrysophora*, *R. rumbolli*, *R. quechua*, and *R. veraguensis*; McCranie et al. 1989, Pramuk & Lehr 2005, Aguayo et al. 2009, Haad et al. 2014). The close relationship between the two clades provides an opportunity to study evolution of endotrophic development within *Rhinella*. Direct observations on breeding ecology are lacking and still needed, but we certainly could expect more variation in the ovipositional and developmental modes than those currently known in the genus.

Other ovipositional modes reported in Bufonidae, but not noticed or confirmed in *Rhinella*, include jelly bars in *Anaxyrus quercicus* (Wright & Wright 1949; but see the discussion in Altig & McDiarmid 2007), rosaries in *Capensibufo rosei* (Channing et al. 2012), and terrestrial clumps in *Oreophrynella nigra* (McDiarmid & Gorzula 1989), *Osornophryne guacamayo* (Gluesenkamp & Acosta 2001), and *Xanthophryne tigerinus* (Biju et al. 2009). Some other species are viviparous, either matrotrophic (*Nimbaphrynoides occidentalis*; Angel & Lamotte 1944) or lecithotrophic (*Nectophrynoides* spp.; see the revision by Liedtke et al. 2014). Descriptions of the breeding biology in poorly-known species of the *R. veraguensis* and *R. acrolopha* groups will contribute with biological data that allow us to understand more thoroughly the diversification in the reproductive biology in *Rhinella* and determine if the shallow clump observed in *R. rumbolli* can have systematic relevance or if it constitutes an autapomorphy of this species.

Clutch infection by Saprolegniaceae

Our report of infection by Saprolegniaceae on eggs of *Rhinella rumbolli* is the first for the genus. Nevertheless, tadpoles of *R. marina* (as *Bufo marinus*) infected with *Aphanomyces* (Saprolegniaceae) were previously reported for two alien populations in Australia (Berger et al. 2001). Other bufonids from which this infection known to affect their eggs and embryos are *Anaxyrus americanus* (Gomez-Mestre et al. 2006, Touchon et al. 2006), *A. boreas* (Blaustein et al. 1994, Kiesecker et al. 2001), *Bufo bufo* (Robinson et al. 2003), and *Epidalea calamita* (Banks & Beebee 1988, Fernández-Benéitez et al. 2008). Further observations coupled with an appropriate experimental design are mandatory to elucidate several aspects related to this fungal infection. First, we are not certain whether the infection is parasitic or a simple colonization of already dead eggs. Epidemiological aspects such as incidence in clutches and eggs, mortality rates, non-lethal effects, etc., should be addressed. Some hints from other bufonids are useful in this regard. Results in *Anaxyrus americanus* reveal that although high percentages of clutches are infected, the mean mortality tends to be low (only 25%), pointing to some defence or palliative mechanism. In some cases, water mould is ingested by larger tadpoles of the same or different species, and the spread of infection throughout the clutch is curbed thus (Gomez-Mestre et al. 2006). Among non-lethal effects, an induced early hatching has been proven in embryos of *A. americanus*, which can bring their hatching forward by up to 36% compared to control embryos (Gomez-Mestre et al. 2006). We observed hatched stage 17–18 embryos in situ, but it is uncertain if this is a regular event in this species, since early hatching is typical of at least some *Rhinella* (e.g., *R. arenarum*: St. 16; del Conte & Sirlin 1951). In the case of *R. rumbolli*, it should be tested whether the high incidence and rapid growth of water mould on its clutches is related to the absence of a jelly tube enveloping the whole clump.

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Appendix 1

Variation in reproductive traits of *Rhinella* spp. All data obtained from clutches, except those indicated with a (from mature ovarian oocytes) or b (clutch and mature ovarian oocytes). * – Data inferred from photographs in publications. Measurements are in mm.

	Oviposition mode	Number of eggs	Ovum pigmentation	Ovum size	String / Strand diameter
***Rhinella acrolopha* group**					
R. acrolopha[a]	?	290 (145 in one side)[1]	unpigmented[1]	2.5[1]	?
R. festae[a]	?	“few”[1]	unpigmented[1]	1.8[1]	?
R. lindae[a]	?	7 + 11[2]	unpigmented[2]	“large”[2]	?
R. macrorhina[a]	?	“few”[1]	unpigmented[1]	2[1]	?
R. nicefori[a]	?	“few”[1]	unpigmented[1]	1.8[1]	?
R. paraguas[a]	?	61[3]	unpigmented[3]	2.24±0.23[3]	?
R. tenrec[a]	?	?	unpigmented[4]	2.5–2.7[4]	?
***R. crucifer* group**					
R. ornata	strings (uniserial)[5,6]	>831 (N=1) (incomplete clutch)[6]	animal pole pigmented[6]	1.84±0.02[6]	3.70±0.25[6]
R. pombali	strings (biserial)[7*]	9952[7]	completely pigmented[7]	?	?
***R. granulosa* group**					
R. azarai	strings (uniserial, scalloped)[6,8]	3770–7548[6,8]	animal pole pigmented[6,8]	1.19±0.06 (1.06–1.33)[6]	1.72±0.19 (1.48–1.97)[6]
R. bergi (as *Bufo pygmaeus*)	strings[9]	?	?	1.00[9]	1.80[9]
R. dorbignyi	strings[10]	?	?	?	?
	strings[10,11]	9000[11]	?	?	3.00–3.50[11]
R. fernandezae (as *B. dorbignyi* in [11])	strings (biserial)[6]	1497[6]	animal pole pigmented[6]	1.13±0.06 (1.02–1.25) 1.29±0.04 (with vitelline membrane: 1.52±0.09)[6]	2.87±0.14[6]
R. merianae (as *B. granulosus* in [12])	strings[12]	983[12]	?	<1.0[12]	?
	strings[13]	900[13]	?	?	?
R. humboldti (as *B. g. beebei* in [14] and *R. granulosa* in [15])	strings[14]	1335–4391[15]	?	1.37±0.10 (1.23–1.55)[15]	?
R. major[a] (as *B. granulosus* in [16] and [17])	?	7784 (1901–13195)[16,17]	pigmented[16,17]	0.52[16,17]	?
	strings (uniserial, scalloped)[6]	6269 (N=1)[6]	animal pole pigmented[6]	0.95±0.07 (0.84–1.07)[6]	1.35±0.03 (1.3–1.39)[6]
R. pygmaea	strings (uniserial)[18]	4140[18]	pigmented[18*]	1.20 (with vitelline membrane: 1.40)[18]	1.60[18]
***R. margaritifera* group**					
R. castaneotica	strings (uniserial)[19*]	61–387[19,20]	pigmented[19*]	?	?
R. ceratophrys[a]	?	1000–1500[21]	pigmented[21]	<1.00[21]	?
R. cristinae[a]	?	“hundreds”[22]	animal pole pigmented[22]	1.20–1.60[22]	?
R. margaritifera (as *B. typhonius* in [23–26])	?	310–2500[23]	animal pole pigmented[23]	<2.00[23]	?
	strings[24]	?	pigmented[24]	“small”[24]	?
	strings[25]	1150–2431[25]	?	1.29[25]	?
	strings[25]	1226[25]	?	1.21[25]	?
	strings[26]	765–2500[26]	pigmented[26]	?	?
	strings[27]	1165–1630[27]	animal pole pigmented[27]	1.30–1.50[27]	?
R. ocellata	strings (uniserial)[28]	2240[28]	pigmented[28]	1.70–1.79[28]	?
R. proboscidea	?	450[13]	?	?	?
	strings (uniserial)[29]	439–473[29]	animal pole pigmented[29]	2.76 (2.4–3.4)[29] (with capsule: 3.7–4.7)[30]	?
R. scitula	strings[31]	?	?	?	?
R. sclerocephala[a]	?	“hundreds, maybe thousand”[32]	animal pole pigmented[32]	1.40–1.80[32]	?
R. stanlaii	?	?	unpigmented[33]	?	?

	Oviposition mode	Number of eggs	Ovum pigmentation	Ovum size	String / Strand diameter
R. sternosignata	?	?	unpigmented[34]	1.4–1.9[34]	?
R. yunga	?	“numerous”[35]	pigmented[35]	“small” [35]	?
***R. marina* group**					
R. arenarum	strings (biserial)[11]	40000[11]	?	?	4.00–5.00[11]
	strings[36]	4000–5000[36]	pigmented[36]	?	?
	strings[37]	33219[37,a]	animal pole pigmented[37,a]	1.06±0.09[37,a]	?
	strings (biserial)[6]	?	animal pole pigmented[6]	1.23±0.22 (with vitelline membrane: 1.40±0.05)[6]	3.15±0.17[6]
R. cerradensis	strings (biserial)[6]	?	animal pole pigmented[6]	2.09±0.08[6]	6.44±0.08[6]
R. icterica	strings[5]	?	?	?	?
R. marina	?	4240–12700[23,a]	animal pole pigmented[23,a]	<1.5[23,a]	?
	strings[12]	8598[12]	?	<1.5[12]	?
	strings[38]	5000–25000[38]	animal pole pigmented[38]	1.7–2.0[38]	?
	strings[13]	4000–10000[13]	?	?	?
	strings[15]	10000–20000[15,b]	?	1.50±0.03 (1.46–1.54)[15,b]	?
	strings[39]	7000–35000[39]	animal pole pigmented[39]	1.5–1.6[39]	?
R. poeppigii	strings[40]	?	?	?	?
R. schneideri (as *B. paracnemis*)	strings[36]	?	pigmented[36]	1.8[36]	?
***R. spinulosa* group**					
R. achalensis	strings (multiserial)[41]	700–2548[41,b]	?	?	?
	strings (multiserial)[42*]	?	pigmented[42*]	?	?
	strings (multiserial)[6]	?	pigmented[6]	?	?
R. amabilis[a]	?	1436[43]	pigmented[43]	0.47 (0.36–0.52)[43]	?
R. arunco	strings[44*]	?	pigmented[44]	?	?
	strings (biserial)[45]	?	pigmented[45]	?	?
R. atacamensis	strings[44]	?	pigmented[44]	?	?
R. spinulosa (as *B. spinulosus papillosus* in [45])	strings (multiserial)[44]	“thousands”[44]	pigmented[44]	?	?
	strings[11]	?	pigmented[11]	1.3 (cf.)[11]	5.00[11]
	strings (multiserial)[46]	3250–6400[46]	animal pole pigmented[46]	1.56±0.09[46]	>8.30[46]
	strings (multiserial)[6]	>1401 (incomplete)[6]	animal pole pigmented[6]	1.64±0.06[6]	5.9±0.18[6]
“*R. veraguensis*” group					
R. chavin[a]	?	266–286[47]	unpigmented[47]	1.82–3.05[47]	?
R. fissipes[a]	?		pigmented[40]	~1[40]	
R. justinianoi[a]	?	?	unpigmented[48]	?	?
R. manu[a]	?	35–40[49]	?	2.00[49]	?
R. multiverrucosa[a]	?	234–290[50]	unpigmented[50]	2.12–2.30[50]	?
R. nesiotes[a]	?	20[51]	unpigmented[51]	1.2[51]	?
R. quechua[a]	?	1014[6]	animal pole pigmented[6]	1.27±0.05 (1.19–1.37) [6]	?
R. rumbolli	shallow clump[6]	2177[6]	animal pole pigmented[6]	1.91±0.09 (with capsule: 3.85±0.36)[6]	–
R. yanachaga[a]	?	136[52]	unpigmented[52]	?	?

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Appendix 2
Examined material

Rhinella azarai – Argentina: Misiones. Departamento Capital: Garupá, Barrio Santa Helena; LGE 3821 (incomplete clutch); LGE 5822 (complete clutch with 5043 eggs); LGE 3736 (complete clutch with 3770 eggs, Blotto et al. 2014); LGE 3737 (complete clutch with 7548 eggs, Blotto et al. 2014); LGE 3738 (complete clutch with 4053 eggs); Departamento Concepción: Concepción de la Sierra; LGE 7329 (incomplete clutch).

Rhinella cf. *cerradensis* – Argentina: Corrientes: Departamento Santo Tomé: Gobernador Virasoro; LGE 6338 (clutches from two different pairs); LGE 6339 (incomplete clutch).

Rhinella fernandezae – Argentina: Corrientes: Departamento Ituzaingó: Ituzaingó; LGE 7008 (incomplete clutch); Entre Ríos: Departamento Islas del Ibicuy: Antigua Ruta Nacional Nº 12; MACN 39247 (female with ovarian oocytes);

Rhinella major – Argentina: Formosa: Departamento Bermejo: Laguna Yema; LGE 7977, 7982 (two incomplete clutches); LGE 8382 (complete clutch with 6269 eggs).

Rhinella ornata – Argentina: Misiones: Departamento San Pedro: Colonia Victoria, Reserva Freaza; LGE 8824 (incomplete clutch).

Rhinella quechua – Bolivia: Carrasco: Departamento Cochabama; MACN 46656 (female with ovarian oocytes).

Rhinella rumbolli – Argentina: Jujuy: Departamento Ocloyas: Río Trigo Pampa; LGE 6415 (complete clutch obtained in the laboratory); LGE 6455, 6461–2, 6471, 6475 (five incomplete clutches infected by Saprolegniaceae).

Rhinella spinulosa spinulosa – Argentina: Jujuy: Departamento Tumbaya: El Angosto, 6 kilometers from El Moreno; LGE 8825 (incomplete clutch); Catamarca: Departamento Andalgalá: Laguna del Río Blanco, 3 km. from Río Candado. FML 07171 (incomplete clutch).