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Esteban O. Lavilla

J. J. Nuñez

F. E. Rabanal

José A. Langone

Rafael O. de Sá University of Richmond, rdesa@richmond.edu

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# The identity of *Rana margaritifera* Laurenti, 1768 (Anura, Bufonidae)

# ESTEBAN O. LAVILLA<sup>1</sup>, ULISSES CARAMASCHI<sup>2</sup>, JOSÉ A. LANGONE<sup>3</sup>,

# JOSÉ P. POMBAL Jr.<sup>2</sup> & RAFAEL O. DE SÁ<sup>4,5</sup>

<sup>1</sup>Fundación Miguel Lillo, Miguel Lillo 251, 4000 Tucumán, Argentina. E-mail: eolavilla@gmail.com
 <sup>2</sup>Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Vertebrados, Setor de Herpetologia, Quinta da Boa Vista, São Cristóvão, 20940–040, Rio de Janeiro, RJ, Brasil. E-mail: ulisses@acd.ufrj.br ; pombal@acd.ufrj.br
 <sup>3</sup>Departamento de Herpetología, Museo Nacional de Historia Natural, Casilla de Correo 399, 11.000, Montevideo, Uruguay. E-mail: pplangone@fcien.edu.uy
 <sup>4</sup>Department of Biology, University of Richmond, Richmond, VA, 23173, USA. E-mail: rdesa@richmond.edu

<sup>4</sup>Department of Biology, University of Richmond, Richmond, VA, 23173, USA. E-mail: rdesa@richmond.edu <sup>5</sup>Corresponding author. E-mail: rdesa@richmond.edu

# Abstract

*Rana margaritifera* was described by Laurenti in 1768 and currently is associated to the genus *Rhinella*, under the combination *Rhinella margaritifera*. Currently, the *R. margaritifera* species group consists of 16 recognized species. Furthermore, many additional species have been suggested to exist in this group which highlights the ambiguity surrounding the identity of *Rhinella margaritifera* and impend further description of the species in this group. After an exhaustive bibliographic review, we concluded that the recent designation of a lectotype for *R. margaritifera* is invalid according with Art. 73, ICZN, 1999. Herein, we designate and provide the description of a neotype for *Rana margaritifera* Laurenti, 1768.

Key words: Amphibia, Rhinella margaritifera, nomenclature, neotype, lectotype, type locality

#### Introduction

Currently, the *Rhinella margaritifera* species group consists of 16 formally recognized species [*Rhinella acutirostris* (Spix, 1824), *R. alata* (Thominot, 1884), *R. castaneotica* (Caldwell, 1991), *R. dapsilis* (Myers & Carvalho, 1945), *R. hoogmoedi* Caramaschi & Pombal, 2006, *R. lescurei* Fouquet, Gaucher, Blanc & Vélez-Rodriguez, 2007, *R. margnussoni* Lima, Menin & Araújo, 2007, *R. margaritifera* (Laurenti, 1768), *R. paraguayensis* Ávila, Pansonato & Strüssmann, 2010, *R. martyi* Fouquet, Gaucher, Blanc & Vélez-Rodriguez, 2007, *R. ocellata* (Günther, 1858), *R. proboscidea* (Spix, 1824), *R. roqueana* (Melin, 1941), *R. scitula* (Caramaschi & Niemeyer, 2003), *R. sclerocephala* (Mijares-Urrutia & Arends, 2001), and *R. stanlaii* (Lötters & Köhler, 2000)]. In addition, there is an unknown, but presumably large, number of cryptic species awaiting description within some of the nominal described species (*e.g.*, Fouquet *et al.* 2007, in a molecular analysis, suggested that *R. margaritifera* species group could consist to up to 11 additional species). Among the 16 nominal species of the group, nine were described in the last decade and, in spite of the renewed interest on documenting the diversity of this group, recent authors have avoided a key known problem while naming new species, i.e., the accurate identity of *Rana margaritifera* Laurenti, 1768, mostly because of the absence of precise type locality and type material.

The name *Rana margaritifera* was included in the synonymy of *Rana typhonia* Linnaeus, 1758, until the latter species was placed in the synonymy of *Rana tigerina* Daudin, An XI [1802] by Hoogmoed (1989), who resurrected a forgotten work by Andersson (1900) (nowadays, *Rana typhonia* is considered as a full species in the combination *Trachycephalus typhonius*; Lavilla *et al.* 2010). Since Hoogmoed (1989) action, the name *Rhinella margaritifera* (Laurenti, 1768) refers to a species complex widely distributed throughout Amazonia and vicinities (Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam, and Venezuela) and extending into Panamá, an extensive distribution for a species which its *terra typica* was explicitly stated as "Brasilia" by Laurenti (1768), following Seba (1734). Aside from its extensive distribution in the Neotropics, this species complex has long

challenge systematists due to the large variation found in adult size, cephalic crests shape and development, sexual dimorphism, etc. (Hoogmoed 1989, 1990).

A first step to clarify the systematics of any cryptic species complex, which is needed to document the existing and currently vanishing biodiversity, is to have a clear concept of the diagnostic characteristics of the population or specimen that bears the name and more precisely place the type locality (de Sá *et al.* 2007).

Herein, to assist in understanding the systematics and diversity of the *Rhinella margaritifera* complex, we (1) analyze the nomenclatural history of this species, (2) refute the designation of a lectotype for the species done by Ávila-Pires *et al.* (2010), and (3) designate a neotype from Northern Brazil for the species described by Laurenti (1768) following article 75.3 of ICZN (1999).

The neotype is designated with the express purpose of clarifying the taxonomic status of the nominal taxon (Art. 75.3.1), is thoroughly described below (Art. 75.3.2-3), and resembles the illustration presented in Seba (1734) of an untraceable individual (Art. 75.3.4, see below) and the character states noted by Laurenti (1768) in the original description of *Rana margaritifera* (Art. 75.3.5). Furthermore, we thoroughly discuss the selected locality (Art. 75.3.6) in a pertinent section, and the individual is housed at the Museu Nacional, Rio de Janeiro, Brasil, in well-known scientific institution (Art. 75.3.7).

#### Material and methods

The first part of this contribution is a bibliographic inquest and, as such, the analyzed materials were [almost] all the texts that, starting with Nieremberg (1635) and ending with Ávila-Pires *et al.* (2010), dealt with *Rhinella margaritifera* under its diverse generic combinations and/or its vernacular names. Only those contributions considered relevant are included in the text.

The second part of this contribution consists in the designation and description of a neotype for *Rana* margaritifera under the combination *Rhinella margaritifera*, based on a specimen from Brazilian Amazonia.

Specimens examined are housed in the Museu Nacional, Rio de Janeiro, Brazil (MNRJ), and are referred in the Appendix. Measurements were taken with digital calipers to the nearest 0.1 mm; abbreviations (in mm) are SVL (snout-vent length), HL (head length), HW (head width), IND (internarial distance), END (eye-nostril distance), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance), TD (tympanum diameter), THL (thigh length), TBL (tibia length), tarsus length (TAL), FL (foot length), and hand length (HAL). Measurements followed Duellman and Trueb (1986) and Napoli (2005). Snout profile terminology followed Heyer *et al.* (1990).

#### Results

#### The identity of Rhinella margaritifera (Laurenti, 1768)

Toads with expanded cephalic ornaments called the attention of travellers and scientists since the beginning of South America explorations. Probably the first references are those of Nieremberg (1635), who described *De bufone Indico* as a toad with "...*Mediocres galeros exaequat*..." (being the *galeros* a kind of ceremonial hat), and Hernandez (1651), who characterized *De Aquaqua* as having "...*galeros mediocres*...". Later, several authors commented on these toads, including Bradley (1721), Vincent (1726), Seba (1734), Klein (1751), and Aubert de la Chenaye des Bois (see Anonymous, 1754), among others. In turn, the peculiar shape of the head of these toads made interesting objects for collectors, and consequently they were frequently found in Natural History Cabinets, as documented by Bradley (1721), who saw "... *in the Cabinets of Mr.* Seba *and Dr.* Ruysh *of* Amsterdam (...) *great Variety of* Frogs *with long* Tails, *curl'd* Ears *and their* Backs *mark'd with various figures*..." Moreover, those with "*curl'd* Ears" from the first Cabinet were published by Seba (1734, plate 71, figs. 6 and 7) and were the foundation for the description of Laurenti (1768), who characterized *Rana margaritifera* as an animal with reddish-brown body, with scattered grains in the form of diluted red pearls, with the lateral region of the body spotted with yellow, the abdomen white with caerulean scattered granules, plus rough feet and hands with four fingers. Additionally, Laurenti described a variety " $\beta$ ." (corresponding to the individual described and depicted by Seba, 1734, in plate 71, fig. 8) as an individual with diluted yellow body, with red pustules, and five fingers on the hand.

Although variety  $\beta$  was traditionally considered as part of the type series of *Rana margaritifera* (Frost 2013,

and literature contained therein), it is not a syntype according to Art. 72.4.1 (ICZN, 1999). The Greek letter " $\beta$ " indicates that Laurenti (1768) considered it a distinct variant, and so did Seba, who described it under the name "*Bufo, Brasiliensis, granis veluti conspersus*", characterized by the presence of five fingers.

Furthermore, both individuals, the one depicted and described in figures 6 and 7 and the one of figure 8 of Seba's (1734) plate 71, were the name-bearers of *Rana mitrata* Houttuyn, 1786. Actually, Houttuyn (1786) used a binomen, expressing: "*Rana Mitrata*\*, *Brasiliensis. Gemyterde Pad als met Pareltjes bexaaid. Seb. Thes. I, Tab. LXXI, Fig. 8.*", being a comma between the asterisk and the geographic reference. The asterisk indicates that it was a new species (in Houttuyn words, "*Asteriscis notatae species ut plurinium novae, mihi determinatae. De nieuwe soorten zyn met Sterretjes getekend.*"). Houttuyn's Catalogue was supressed for nomenclatorial purposes under plenary powers, by the International Commision of Zoological Nomenclature (Opinion 380 – January 24<sup>th</sup>, 1956; Hemming, 1956), based on a proposition of Engels (1952). Consequently, the individual depicted in figures 6 (dorsal) and 7 (ventral) of plate 71, and characterized in page 114 under the name "*Bufo, Brasiliensis, Margaritis veluti conspersus,* Aquaqua *dictus*" (Seba 1734), is the holotype by monotypy (see provisions of Art. 73, ICZN, 1999) of *Rana margaritifera* Laurenti, 1768, invalidating its designation as lectotype by Ávila-Pires *et al.* (2010). Besides, even if that action was valid, it would be innocuous referring the identity of *Rana margaritifera*, since the figured specimen, and purportedly lectotype, is currently lost and the associated type locality is imprecise.

Only two character states provided in the diagnosis of *Rana margaritifera* by Laurenti (1768), the presence of pearl-shaped warts (*granulis margaritiformibus*) and the presence of rough feet (*pedibus hispidus*), are informative. Following Art. 72.4.1.1 (ICZN, 1999), and for a more accurate understanding of the species, this characterization can be enriched with some character states taken from Seba (1734), including (1) the triangular shape of the head, provided with a quadrangular ornament, similar to a priest hat; (2) the dorsal pattern with a noticeable white middorsal band; (3) the presence of a white band on both sides of the head and body; (4) the triangular shape of throat in ventral view, (5) surrounded by a white margin; and (6) the presence of dark, pearl-shaped granules scattered over the ventral region.

During the XVIII Century, this individual was cited several times, tied either to *Rana margaritifera* [*i.a.*, Daubenton (1784), Gmelin (1788), Lacépède (1788; An VII), and Bonnaterre (1789, who even copied Seba's figure 6, pl. 71 in his Pl. 4, fig. 1)] or related to *Rana typhonia* (*non* Linnaeus, 1758) [Fortia de Piles (1796), Krünitz (1778), and Schneider (1799)].

Schneider (1799), under the name "VI. Typhonius", provided a detailed redescription, diagnosing the species as: "Margo capitis aucta membrana altra supra oculos et aures eminente, dorsi medii et laterum ordine triplice callorum acutorum". In this way, Schneider (1799) precised the Linnean "auricularibus lobis ovatis" as an "aucta membrana altra supra oculos et aures eminente", referring clearly to a toad with expanded cephalic crest. In the comment on the original description, Schneider also stated "... notitiae auctorem Rolandum nominans. Habitare in America ...", but actually the reference to Rolander regards to Trachycephalus typhonius (Lavilla et al. 2010).

According to Schneider (1799), the name Rana margaritifera included: (a) Bufo, Brasiliensis, Margaritis velutis conspersus, Aquaqua dictus (pl. 71, figs. 6–7) and Bufo, Brasiliensis, granis velutis conspersus (pl. 71, fig. 8) of Seba (1734); (b) Bufo margaritifer of Laurenti (1768); (c) the "Grenouille perlée" (= R[ana] Gemmata) and the "G[renouille] Typhone" (= R[ana] Typhonia) of Bonnaterre (1789, who already included Seba's and Laurenti's references); (d) Rana mitrata brasiliensis (sic) from Mus. Huttunianum I. p. 19. N° 118 and 119 (Houttuyn, 1786); (e) the toads identified as N°s 14 and 15 in Levini Vincentii Musei (described in Vincent, 1726), and (e) a series of specimens unidentified by Schneider from Museo Electorali Dresdensi, Musei Lampiani, and Musei Ducalis Brunovincensis. The common thread among all these taxa is that they are all from South America and may correspond to diverse populations of Rhinella.

In the same book, Schneider (1799) described a second species, today in the synonymy of *Rhinella* margaritifera. Under the epithet "XI. Nasutus", he diagnosed the taxon as: "Rostrum obtusum nares utrinque appositas gerit; ab orbitae margine alto plica elata ad parotidem, altera ad occiput excurrit." The species was based on (1) individuals 297 and 320 of the Museum Linckiani (described in Linck, 1783) and (2) on Seba's (1734) "Bufo, Brasiliensis, minor; maculatus." The synonymy of this taxon with Rana margaritifera originated with Daudin (An XI) ["...Le Crapaud Nasique (Bufo nasutus) décrit par Schneider dans son ouvrage latin sur les Amphibiens, est certainement le synonyme du Crapaud Perlé, car il a vu un pli élevé sur chaque oeil, et prolongé jusqu'aux parotides..."].

Unfortunately, the individual depicted by Seba (1734) in figures 6 and 7 of plate 71 is not traceable and, as far

as we know, the material from Seba's second collection, the one that was the base of his *Thesaurus*, was dispersed after an auction in 1752 [the individual is neither at Vienna Museum (Aaron Bauer, per. com.), nor in Paris (Thireau *et al.* 1998) and there are no clues in the sale's catalogue of Seba's specimens (Anonymous, 1752), nor in Boeseman (1970), or Engels (1937, 1961)]. This situation, plus the conflictive attributions of the name to a concrete biological population, led us to the conclusion that a designation of a neotype is needed for the Laurenti's (1768) species. With this goal in mind, we did an extensive search in the literature and examined every description we were able to find of toads with expanded cephalic crests, reported either as *Rana typhonia (non* Linnaeus, 1758) or *Rana margaritifera*, plus their diverse generic combinations and synonyms. Subsequently, following Seba (1734) and Laurenti (1768) stated provenance, we focused on those from Brazil, as the type locality was originally established as "*Brasilia*" by both authors.

#### The figures of Rana margaritifera in Seba (1734)

The Dutch apothecary and businessman Albertus Seba (1665–1736) used much of his time, money, and connections to acquire natural history specimens and to make extensive private collections, generally called "cabinet of curiosities." In 1717, Seba sold his first collection to Peter, the Great, Tsar of Russia, and immediately set out to build a second (Engel 1937). This second collection, which would come to be larger than the first, was the basis for the production of the *Thesaurus* (Bauer 2002). The seminal work of Seba was published in four volumes appearing in 1734, 1735, 1750, and 1765 (Holthuis 1969), being the volume II almost completely herpetological.

A general problem affecting early natural history collections was, and ever today is, the fixation, conservation, and storage of specimens. Although dry and hard materials, such as calcareous mollusc shells, do not present major decay or preservation problems, other natural specimens, mainly those maintained in some wet preservative, required complex conservation measures. Many of these procedures were borrowed from other disciplines, mainly from Medicine and its methods for preservation of corpses (Müsch 2001). Besides not mentioned by Seba, the depicted amphibians, as well as fishes and reptiles, surely faced preservation problems. The specimen received by the artist to draw normally was preserved in an alcoholic fluid, the "wine spirit", more likely a brandy. The specimen generally compressed, contorted, often faded and partially decomposed, immersed in a murky fluid, was transformed into very clear and distinct illustration. Obviously, the elements important for the identification of the animal were rendered better and considerably more distinct in the illustration than in the actual specimen (Müsch 2001). Subsequently, the illustration was transferred to engraved copper plates. For this purpose, the engraver received an additional copy of the drawing in mirror image, so that the final print would come out the proper way (but in some cases such copies were not made and the pictures were produced in reverse) (Willmann & Rust 2001). Seba himself supervised the preparation of nearly all engravings. He utilized no less than 13 artists and the differences in techniques and quality can be seen in the works (Müsch 2001; Bauer 2002).

The expensive work was initially published in black-and-white. It is not known whether the publishers also offered hand-painted editions, but it is more probable that the buyers had to order the painting at their own expenses. An unknown number of specialist colourists painted an unknown number of copies. The colours add attractiveness to the plates, but their purpose was not just aesthetic enhancement, as they had a scientific use as well (Müsch 2001). It is not known if the colourists had access to the specimens, but the direct observation of specimens at all would provide minimal information about coloration, particularly in wet-preserved ones, like in amphibians. The colourist's skill and knowledge of the subject played an important part in enabling the illustration to convey their message (Müsch 2001). However, facing faded specimens probably the colourist adapted the colour from other known species (*e.g.*, a South American amphibian could receive the colour pattern of an European one) or added colours for purely aesthetic effect.

Figures 6 and 7 of plate 71 in volume I of the *Thesaurus* (Seba 1734) depict the dorsal and ventral views, respectively, of the species called *Rana margaritifera* by Laurenti (1768). As indicated above, there are versions in black-and-white (Fig. 1 A) and hand painted (Fig. 1 B, C). Apparently a female specimen was figured, as it does not present any darkening on gular and shoulder regions, a very conspicuous male character (young and adults; UC personal observation) and which surely the artist would represent. Cephalic crests are depicted, from the supratympanic region to the tip of snout; posteriorly, the supratympanic crest is followed, without interruption, by a dorsolateral line of spinulose tubercles; in coloured versions, these crests and tubercles lines are bordered by a

white line, but probably this was only a way to delimit the crests, and not an actual white line in the specimen. The eye is small and the tympanum was not figured. The general dorsal aspect shows an immaculate background, white or reddish brown depending the version of the work (Fig. 1 A–C). Presence of a mid-dorsal stripe, from snout to vent, white or cream coloured; on the anterior part of the body this stripe has 7–8 details emphasizing the emerging dorsal vertebral apophyses (Fig. 1 A); in coloured versions, these apophyses are red (Fig. 1 B–C), a character not seen in actual specimens. The posterior corner of the mouth is produced into a rounded tubercle. The arm is uniformly white and the forearm is dorsally dark (Fig. 1 A); in coloured versions, there are two different interpretations, with the arm uniformly reddish brown (Fig. 1 B) or yellow cream with reddish brown blotches (Fig. 1 C). The legs are uniformly white, with only a suggestion of two darker transverse bars on the right shank (Fig. 1 A); in coloured versions, the thigh, shank, and foot have yellow cream background with four transverse reddish brown bars on thigh, five transverse reddish brown bars on shank, and six transverse reddish brown bars on foot (Fig. 1 B), or there are only poorly defined transverse reddish brown bars and spots on legs (Fig. 1 C). Ventral views show uniform white or cream surfaces, with small and scattered black or blue dots, without a defined pattern.

Although considering that the colours associated to the figures are of little confidence, three main morphological characters can be utilized to define *Rana margaritifera*: (1) the presence of cephalic crests, from the supratympanic region to the tip of the snout; (2) posterior corner of mouth with a rounded tubercle; and (3) the presence of emerging dorsal vertebral apophyses on dorsum. Considering these characters, a neotype was designated for the species (see below).

#### Determination of a neotype for Rhinella margaritifera

As noted in the Introduction, there are 16 currently accepted names applied to Neotropical toads with more or less expanded cephalic crests (Frost 2013) (*Rhinella martyi* Fouquet *et al.* 2007 was considered a junior synonym of *R. margaritifera* by Ávila-Pires *et al.* 2010). However, *Rhinella margaritifera* (Laurenti, 1768) is the only taxon that lacks a tangible name-bearing type specimen and the only taxon with an extremely imprecise type-locality ("Brasilia", referring to the country name Brazil, not to the current city/state of Brasília).

In order to eliminate the possibility that one of the available names could fit with the descriptions of the namebearing type (Seba 1734; Laurenti 1768), we compared Seba (1734) figures and Laurenti (1768) descriptions and geographic distribution with the available names of toads with cephalic crests. Through this re-analysis it became evident that all the character states established for *Rhinella margaritifera* (Laurenti, 1768) fit into the variation of several species included in the *R. margaritifera* group. In consequence, we selected a specimen from Brazilian Amazonia, by considering that the specimen depicted by Seba (1734) would have the greatest possibility to come from that region.

#### The type locality of Rana margaritifera Laurenti, 1768

In describing his *Rana margaritifera*, Laurenti (1768) clearly stated its type locality for "Brazil" ("*Habitat in Brasilia*"), following the information of Seba (1734) who stated that the figured specimen in the plate 71, figures 6 and 7, came from "Brazil, once more through Portugal" ("*ex Brasilia, nuperque etiam ex Portugallia*"). Although very extensive, this statement excludes other possible origins of the Seba's specimen, mainly Suriname, a well-known source of his materials.

The specimens utilized by Seba in the "*Thesaurus*" were gathered in his second collection (see above on first collection). The very busy port of Amsterdam with ships coming in from many far away regions, offered Seba excellent opportunities to assemble the objects for his "cabinet of curiosities" (Holthuis, 1969). However, the specimen at issued was received through Portugal from Brazil, a very secluded colony in the beginning of the XVI–XVIII centuries, with only three authorized ports in Belém, Salvador, and Rio de Janeiro. The first one, Belém, was the port of entry and way out of Amazonia, and the other two were located in the east coast of Brazil, in the Atlantic Forest region. All three ports are potential candidates as original departing points of Seba's specimen, with great influence on its identification.

As seen above, possibilities for sending minerals and goods, and for instance natural history specimens, from

the colony of Brazil to Portugal in the XVII and XVIII centuries were the ports of Salvador and Rio de Janeiro, currently in the states of Bahia and Rio de Janeiro, respectively. If the specimen figured in Seba (1734) had come from one of these ports in the Atlantic Forest region then it would belong to the species currently known as *Rhinella hoogmoedi* Caramaschi & Pombal, 2006. Nevertheless, this species differs from that figured in Seba (1734) because it: a) has small, poorly developed supra-orbital crest and canthus rostralis provided only by a low canthal crest; b) the dorsolateral line of tubercles is not continuous to the supra-orbital crest, which is separated from the parotoid gland; c) the tubercles on the posterior corners of mouth are small, not seen from above; d) the vertebral apophyses are not salient on dorsum; and e) only four poorly developed mid-dorsal tubercles are present (see figs. 1–2 in Caramaschi & Pombal 2006). Based on these characters, an Atlantic Forest origin for the specimen of Seba was disregarded. On the other hand, the Seba's (1734) figure fully agrees with specimens from an Amazonia population and we conclude that this is the most probable origin of the Seba's "*Bufo, Brasiliensis, Margaritis veluti conspersus*, Aquaqua *dictus*".

Amazonia was known since Vicente Yáñez Pinzón (1462–1514) discovered Brazil in January 1500; however, it was officially discovered by Pedro Álvares Cabral on 22 April 1500 for the Portuguese Crown. As a matter of fact, Pinzón obtained a female opossum (*Didelphis*, a marsupial mammalian of the family Didelphidae) with younglets, in the mouth of the Amazon River; this was the first American animal described by an European, as "*Animal monstrosum*" (Papavero & Teixeira, 1999). Since then, several expeditions, mainly in search of gold, precious stones, slaves, and for religious purposes, entered Amazonia through the main rivers. Amphibians were poorly represented in older records; for example it is possible to refer to Dom Lourenço Álvares Roxo de Potflis (1699–1756) who, in 1752, cited the "*cunauaru-icica*" ["*cunauarúicica*" in the original; it refers to the resin of the "*cunauaru*" frog, *Trachycephalus resinifictor* (Goeldi, 1907) (Hylidae); Teixeira *et al.* 2010]. Besides these scanty citations, it is very feasible that amphibian specimens were captured and sent to Portugal through the port of Belém (established in 1616). In view of this, we selected as the neotype for *Rana margaritifera* Laurenti a specimen from Brazilian Amazonia that agrees with the figure presented by Seba (1734) and with Laurenti's (1768) description.

The choice of a specimen as neotype for *Rana margaritifera*, and consequently the restriction of its type locality, was based on the following premises. First, the specimen would come from an Amazonian locality known to exist at the end of the XVI Century or beginning of the XVII Century, to be in temporal accordance to the specimen figured by Seba (1734). Second, the specimen would fit the three main morphological characters that can be utilized to define Rana margaritifera figured in Seba (1734): (1) presence of cephalic crests, from the supratympanic region to the tip of the snout; (2) posterior corner of mouth with a rounded tubercle; and (3) presence of emerging dorsal vertebral apophyses. And three, the neotype would comes from a population currently known as *R. margaritifera* (or from a currently unnamed population), in order to avoid additional taxonomic chaos by leading to additional synonymies. Specimens from Central Amazonia, mainly obtained along the Solimões-Amazonas Rivers complex, were excluded because did not fulfil the second or the third premises (threatening the currently known R. acutirostris, R. dapsilis, R. magnussoni, R. proboscidea, and R. roqueana); the same was considered for the Xingu River, in the State of Pará (threatening *R. castaneotica*); the remaining species have extra-Amazonia occurrences. Consequently, a specimen from Humaitá, State of Amazonas, Brazil, was selected as neotype for R. margaritifera (see below) because: (1) the region of the currently known Municipality of Humaitá, on the Madeira River left bank, was colonized since a Royal Letter from March 19, 1693 designated the Jesuit priests to catechise the natives from that region (IBGE, 1957). Furthermore, the Madeira River was an important way from the inland city of Cuiabá, in the State of Mato Grosso, to Manaus, in the State of Amazonas, to Belém, in the State of Pará, and to the Atlantic Ocean. Travellers probably stopped in that incipient colonization point; consequently, it is not implausible that specimens from the Humaitá region could have reached Portugal through the port of Belém. (2) The selected specimen fits the morphological characters depicted in the Seba's (1734) figure (see description of the neotype below). And (3), the population in the region of Humaitá is currently identified as R. margaritifera and will not threaten the validity of any of the other nominal species in the group. Unfortunately, there are no tissues for molecular analysis available for the neotype or from specimens from the designated type locality.

In summary, we assigned a neotype for *R. margaritifer* Laurenti from the locality of Humaitá, State of Amazonas, Brazil, a population that has not been associated with other taxa in the *R. margaritifer* species complex and which fully agreed in morphology with the original description. We recognize *R. margaritifer* as a complex of cryptic species across this large geographic region but we consider the placement of *R. martyi* in the synonymy of

*R. margaritifer* by Avila-Pires *et al.* (2010) premature. Indeed, we aim to facilitate the documentation of the hidden variation in this group by providing type material and associated locality data for the type species in this large species complex. Newly collecting tissue samples for molecular analysis in the area around Humaitá, as well as across the species geographical range, will be an important next step to understand the diversity of this taxonomically convoluted group of toads.

# Rhinella margaritifera (Laurenti, 1768)

Rana margaritifera Laurenti, 1768: 30.

**Neotype:** MNRJ 71538, adult female (Figs. 2–3), collected in the Municipality of Humaitá (07°30'S, 63°01'W; approx. 60 m a.s.l.; datum WGS84), State of Amazonas, Brazil, on 12 April 1985 by F.L. Franco and B.V.B. Aloise.

**Description of the neotype:** Body robust; head slightly wider than long, head length 89.8% of head width; head length 31.1% of SVL; head width 34.6% of SVL. Snout mucronate in dorsal view (Fig. 3A); in profile (Fig. 3B), nearly acute. Top of snout and head slightly concave; canthus rostralis well defined by the canthal crests, curved; loreal region concave. Nostrils lateral, protuberant, slightly directed dorsally and backwards, nearer to the tip of snout than to eyes; internarial distance smaller than the eye-to-nostril distance, eye diameter, upper eyelid width, and tympanum diameter; eve-to-nostril distance slightly larger than the eve diameter, and larger than the tympanum diameter and upper eyelid width; eye diameter larger than the upper eyelid width and tympanum diameter; upper eyelid width 39.5% of interorbital distance. Canthal, and supra-orbital crests developed, parietal crest poorly developed; post-orbital crests large, forming conspicuous lateral ledges; distance of extremities of the post-orbital crests larger than head width. Tympanum large, round, with a distinct annulus; tympanum diameter 89.2% of eye diameter. Parotoid glands, in dorsal view, small, triangular, elongated; in lateral view, elliptical, continuous to the post-orbital crest; parotoid gland length slightly larger than the post-orbital crest length. External border of the parotoid gland with a line of pointed tubercles which continues along the lateral side of body to the groin. Five vertebral apophyses salient on dorsum. Lips not flared; eyes visible from below. A Vshaped incision in the maxilar symphysis; a rounded tubercle at the posterior corner of mouth. Vocal sac and vocal slits absent. Choanae small, lateral, widely separated; tongue large, two times as long as wide, free and not notched behind.

Forelimbs robust, forearms as robust as arms; a line of small pointed tubercles along the lateral border of forearm. Hand (Fig. 3C) with medium-sized, slender fingers not webbed, in crescent order of size, IV < II < I < III; lateral fringes poorly developed, formed by a line of spinulose tubercles. Fingers tips slightly expanded, smooth, posteriorly delimited on the dorsal and ventral faces by a groove. Palmar tubercle large, rounded, flat, smooth; thenar tubercle approximately one fourth of the palmar tubercle, ovoid, flat, smooth. Subarticular tubercles developed, conical, unique. Supernumerary tubercles of varied sizes, distinct, conical, irregularly distributed on the ventral surfaces of hand and fingers.

Hindlimbs short, robust. Tibia length smaller than the thigh length; tibia length 87.7% of thigh length and 37.3% of the SVL; thigh length 42.6% of SVL; sum of tibia and thigh lengths 80.6% of the SVL; tarsus-foot length larger than the tibia and thigh lengths, 52.9% of the SVL. A line of conical, spinulose tubercles along the posterior borders of tarsus. Foot (Fig. 3D) with short toes, moderately robust, in crescent order of size, I < II < V < III < IV; toes not webbed, but extensively fringed. Lateral fringes of toes with a line of spinulose tubercles. Tips of toes in small rounded bulbs, smooth, posteriorly delimited on the dorsal and ventral faces by a groove. Outer metatarsal tubercle small, rounded; inner metatarsal tubercle large, approximately three times the outer, ovoid, with the external border free. Subarticular tubercles small, conical, unique. Supernumerary tubercles distinct, conical, unequal in size, approximately aligned on the ventral surfaces of foot and toes.

Skin on dorsum and limbs granulose, with many tubercles small, rounded, irregularly distributed without forming a defined pattern. Ventral surfaces finely granulose, with many slightly larger granules scattered over the entire surface.







**FIGURE 1.** *Rana margaritifera* figured in Seba (1734), plate 71, figures 6 and 7. (A) Black-and-white copy of the Missouri Botanical Garden, Saint Louis, Missouri (USA), available at <<u>http://www.animalbase.org</u>>. (B) Hand painted reprinted from the copy of the Koninklijke Bibliotheek, The Hague, Netherlands, by Taschen, Köln. (C) Hand painted copy of the Museu Nacional, Rio de Janeiro, Brazil.



FIGURE 2. Dorsal and ventral views of Rhinella margaritifera, neotype (MNRJ 71538, SVL 70.7 mm).

Color of neotype: In preservative, dorsum and laterals of body and limbs uniformly dark grayish brown; a clear brown stripe on dorsum, on the vertebral column, from the tip of snout to vent. Throat and chest gray, with white tubercles irregularly distributed. Belly and ventral surfaces of hindlimbs and forelimbs uniformly clear cream with gray vermiculations and scattered cream tubercles. Proximal ventral surface of thighs gray with white granules. Posterior surface of tarsus to the ventral surface of toes IV and V, gray. Iris yellow, with dense black vermiculations.

Measurements of neotype (mm): SVL 70.7; HL 22.0; HW 24.5; IND 4.3; END 6.7; ED 6.5; UEW 4.7; IOD 11.9; TD 5.8; THL 30.1; TBL 26.4; TAL 14.5; FL 22.9; HAL 16.6.

**Remarks:** The region of Humaitá was succinctly described by Gottsberger (1978), Caramaschi & Jim (1983), and Caramaschi & Cruz (2001). Humaitá is a small town on the left bank of the Madeira River, 51 m above sea level. It is surrounded on the western side by the far-extending Puciari-Humaitá savannas. To the north and south is the large dry or "terra firme" forests, frequently crossed by small rivers, regionally called "igarapés". Along these streams are the flooded areas with the "várzea" and "igapó" forests. These small rivers of the region drain the local savannas, and bring black water (Sioli 1975). During the dry season, when the water is at its lowest level, the streams are generally narrow, about 1–2 m wide and extensively surrounded by the "várzea" forest. At the rainy season, the white water (Sioli 1975) of the Madeira River invades the "igarapés" about 500–800 m upstream from its mouth, and it is here that a sharp interface between the white Madeira and black "igarapé" waters occurs. At the highest water level, about 10–12 m above the lowest, the "igarapés" are about 100 m wide, invading laterally the "várzea" forest, and it is bordered directly in both sides by the "terra firme" forest. The vegetation of the "várzea" and "terra firme" forests in the region seems to be reasonably undisturbed. Even at the highest water level, most of the tree crowns emerged 5–10 m, and in many cases huge tree trunks rose 15–20 m above the water surface. On the soil of both "várzea" and "terra firme" forests is a thick leaf litter cover, where specimens of *Rhinella margaritifera* were collected.

The presence of many color patterns is common among the members of the *Rhinella margaritifera* species group (see, for example, Caramaschi & Niemeyer 2003; Caramaschi & Pombal 2006). Among specimens obtained at the current type locality of *R. margaritifera*, several patterns of dorsal color were obtained (Fig. 4). The general

color varies from brownish cream, grayish brown, to grayish red. The mid-dorsal longitudinal line may be absent (four specimens), narrow (three specimens), medium (one specimen), and wide (four specimens). Dark brown spots on dorsum, lateral to mid-dorsal line, may be absent (four specimens) or present, in variable number, localization, size, and shape (nine specimens).



FIGURE 3. *Rhinella margaritifera*, neotype (MNRJ 71538, SVL 70.7 mm). (A) Dorsal and (B) lateral views of head; ventral views of (C) hand and (D) foot.

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FIGURE 4. Variation in dorsal color patterns of *Rhinella margaritifera*. (A) MNRJ 71538, Humaitá, neotype. (B) MNRJ 71539, Humaitá. (C) MNRJ 28674 Humaitá–Porto Velho Road. (D) MNRJ 71549, Humaitá. (E) MNRJ 71553, Humaitá. (F) MNRJ 71551, Humaitá. (G) MNRJ 71547, Humaitá. (H) MNRJ 71548, Humaitá. (I) MNRJ 28673, Humaitá–Porto Velho Road. (J) MNRJ 71552, Humaitá. (K) MNRJ 71550, Humaitá. (L) MNRJ 28675, Humaitá–Porto Velho Road.

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#### APPENDIX. Specimens examined.

- *Rhinella margaritifera*: BRAZIL: Amazonas: Humaitá, Km 49 of the Humaitá–Porto Velho Road (MNRJ 28673–28675); Humaitá (MNRJ 71538, neotype; MNRJ 71539, 71547–71553).
- *Rhinella hoogmoedi*: BRAZIL: Bahia: Canavieiras, Fazenda Santa Clara (MNRJ 40325, holotype; MNRJ 40326-40331, 40508-40510, paratypes).