Caldasia **42(2):171-180** | **Julio-diciembre** 2020
http://www.revistas.unal.edu.co/index.php/cal

CALDASIA
Fundada en 1940
ISSN 0366-5232 (impreso)
ISSN 2357-3759 (en línea)

ZOOLOGY

# First record of *Vitreorana ritae* (Anura, Centrolenidae) for southern Amazonia inferred from molecular, reproductive and acoustic evidence

## Primer registro de *Vitreorana ritae* (Anura, Centrolenidae) en el sur de la Amazonía inferida a partir de evidencia molecular, reproductiva y acústica

Marcos Penhacek[1*] | Samuel Anjos[1,2] | Elciomar Oliveira[3] | Emil José Hernández-Ruz[4] | Luís Reginaldo Rodrigues[5] | Vinícius Guerra[6] | Domingos Rodrigues[1,2]

- **Received:** 9/jul/2019
- **Accepted:** 24/feb/2020
- **Published online:** 13/mar/2020

**Citation:** Penhacek M, Anjos S, Oliveira E, Hernández-Ruz EJ, Rodrigues LR, Guerra V, Rodrigues D. 2020. First record of *Vitreorana ritae* (Anura, Centrolenidae) for southern Amazonia inferred from molecular, reproductive and acoustic evidence. Caldasia 42(2):171–180. doi: https://dx.doi.org/10.15446/caldasia.v42n2.79487.

## ABSTRACT

Frogs of the family Centrolenidae are adapted to live at high altitudes, and so it is not surprising that their greatest diversity is in the Andes mountain range. Here we extend the known geographic distribution of *Vitreorana ritae* by more than 550 km towards southern Amazonia. The species was identified by morphological, acoustic and molecular characteristics (mitochondrial marker 16S rRNA). The records reported here are the first for the genus *Vitreorana* in the state of Mato Grosso, Brazil, as well as for the transition zone between the Amazonia and Cerrado biomes. These records are consistent with the hypothesis that the low number of species of centrolenids reported in the lowlands of the Amazon region may be the result of limited sampling. Even though *V. ritae* is distributed throughout the Amazon, most of its diagnostic morphological characteristics are conserved. Thus, the great rivers of the Amazon Basin do not seem to act as geographical barriers for this species; however, due to the limited sample size, further acoustic and molecular studies are needed to confirm this hypothesis.

**Keywords.** Acoustic parameters, Amazon-Cerrado ecotone, glass frog, intraspecific genetic variation

---

[1] Instituto de Ciências Naturais, Humanas e Sociais e Acervo Biológico da Amazônia Meridional – ABAM, Universidade Federal de Mato Grosso, Campus de Sinop. Av. Alexandre Ferronato, 1200, CEP 68550-000, Distrito Industrial, Sinop, Mato Grosso, Brazil. penhacek@yahoo.com.br*, samuelherpeto@yahoo.com.br, djmingo23@gmail.com

[2] Programa de Pós-Graduação em Ciências Ambientais, Instituto de Ciências Naturais, Humanas e Sociais, Universidade Federal de Mato Grosso, Campus de Sinop. Av. Alexandre Ferronato, 1200, CEP 68550-000, Distrito Industrial, Sinop, Mato Grosso, Brazil.

[3] Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede BIONORTE, Universidade Federal do Amazonas, Av. Gen. Rodrigo Octávio Jordão Ramos, 3000, CEP 69077-000, Manaus, Amazonas, Brazil. elciomar.atractus@gmail.com

[4] Programa de Pós-Graduação em Biodiversidade e Conservação, Campus Universitário de Altamira, Universidade Federal do Pará, Rua Coronel José Porfírio, 2515, CEP 68372-040, Altamira, Pará, Brazil. emilhjh@yahoo.com

[5] Programa de Pós-Graduação em Biociências, Universidade Federal do Oeste do Pará, Rua Vera Paz, s/n (Unidade Tapajós), CEP 68035-110, Santarém, Pará, Brazil. luisreginaldo.ufpa@hotmail.com

[6] Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Goiás (UFG), Campus Samambaia, CEP 74001-970, Goiânia, GO, Brazil. vinicius.guerrabatista@gmail.com

* Corresponding author

## RESUMEN

Las especies de anfibios de la familia Centrolenidae están adaptadas a vivir en regiones de elevada altitud, por lo que no sorprende que su mayor diversidad ocurra en la Cordillera de los Andes. Aquí expandimos el ámbito de distribución de *Vitreorana ritae* por más de 550 km al sur del río Amazonas. La identificación de la especie se realizó con base en la comparación de las características morfológicas, acústicas y moleculares (marcador mitocondrial 16S rRNA). Nuestro registro es el primero de este género para el Estado de Mato Grosso, Brasil, así como para la zona de transición entre los biomas Amazonía y Cerrado. Este registro es concordante con la hipótesis de que el bajo número de especies registradas en la planicie amazónica puede ser el resultado de un menor esfuerzo de muestreo. Observamos que, a pesar de la amplia distribución de la especie en la Amazonía, la mayoría de las características morfológicas diagnósticas de *V. ritae* se conservan. Sugerimos que los grandes ríos de la cuenca amazónica parecen no actuar como barrera geográfica para esta especie. Sin embargo, y como resultado de nuestro pequeño tamaño de muestra, se requerirán estudios acústicos y moleculares adicionales para confirmar esta hipótesis.

**Palabras clave.** Ecótono Amazonía-Cerrado, parámetros acústicos, rana de cristal, variación genética intraespecífica

## INTRODUCTION

The family Centrolenidae comprises 157 species of eleven genera of what is commonly referred to as glass frogs (Guayasamin *et al.* 2009). They are distributed from the tropics of Central America, throughout the tropical Andes, the Sierra Nevada in Colombia, the coast of Venezuela to Bolivia, Trinidad and Tobago, the Guiana Shield, and the Atlantic Forest of southeastern Brazil and northeastern Argentina (Frost c2019). Fourteen centrolenid species are currently known to occur in Brazil: *Hyalinobatrachium cappellei* Van Lidth de Jeude, 1904; *H. carlesvilai* Castroviejo-Fisher, Padial, Chaparro, Aguayo-Vedia, and De la Riva, 2009; *H. iaspidiense* (Ayarzagüena, 1992); *H. mondolfii* Señaris and Ayarzagüena, 2001; *H. muiraquitan* Oliveira and Hernández-Ruz, 2017; *H. munozorum* (Lynch and Duellman, 1973); *Teratohyla adenocheira* (Harvey and Noonan, 2005); *T. midas* (Lynch and Duellman, 1973); *Vitreorana baliomma* Pontes, Caramaschi, and Pombal, 2014; *V. eurygnatha* (Lutz, 1925); *V. franciscana* Santana, Barros, Pontes, and Feio, 2015; *V. parvula* (Boulenger, 1895); *V. ritae* (Lutz, 1952); and *V. uranoscopa* (Müller, 1924) (Frost c2019).

Although the number of Brazilian centrolenid species has increased in recent years with new records for previously isolated sites, mainly in the Amazon (ICMBio c2009, Toledo *et al.* 2009, Venâncio *et al.* 2014), there still remain large knowledge gaps regarding the distribution of several species, most of which are known from isolated localities within a large geographic range (Frost c2019). Moreover, only 79 % of Brazilian centrolenids have had their advertisement call described (Guerra *et al.* 2018). Specific information about the distribution and vocalization of anuran species is of great importance for taxonomic and conservation purposes (Castroviejo-Fisher *et al.* 2011, Guerra *et al.* 2018). Bioacoustic information has been particularly relevant in studies of anuran behavior (Gerhardt and Huber 2002), community ecology (Oseen and Wassersug 2002) and evolution (Cocroft and Ryan 1995, Goicoechea *et al.* 2010), and has contributed to the discovery of many morphologically-conservative cryptic species (Padial *et al.* 2008, Glaw *et al.* 2010).

*Vitreorana ritae* (Lutz, 1952) occurs in eastern Ecuador, Colombia, Peru, southern Guyana, eastern Suriname, French Guiana and from the state of Roraima south to central Amazonia and west to extreme western Amazonas

State in Brazil (Frost c2019). The species has been recorded at different localities in the state of Pará, such as the Caxiuanã and Tapajós National Forest (Gonçalves 2013), the Xingu River (Vaz-Silva *et al.* 2015), and the Altamira National Forest (ICMBio c2009). *Vitreorana ritae* is the senior synonym of *Cochranella oyampiensis* (Lescure, 1975) and *Cochranella amertasia* (Flores, 1987). It is morphologically similar to *V. helenae*, which is considered its sister species (Kok and Castroviejo-Fisher 2008, Castroviejo-Fisher *et al.* 2014, Moraes *et al.* 2017) and occurs in the Tepuis of the Guiana Shield, although *V. ritae* is distinguished by its gray iris and green dorsum with black spots (yellow and uniformly green dorsum, respectively, for *V. helenae*; Guayasamin 2008).

The present study extends the distribution of *Vitreorana ritae* with sites in the municipalities of Cotriguaçu and Sinop in the state of Mato Grosso, Brazil. The advertisement call and tadpole morphology and molecular data from a male collected in the transition zone between the Cerrado and the Amazon are compared with that of previously known populations.

## MATERIAL AND METHODS

### Data collection and species identification

A female of *Vitreorana ritae* (SVL = 22.9 mm) was collected on 20 December 2015 on the São Nicolau Farm in the municipality of Cotriguaçu, state of Mato Grosso, Brazil (09º 50' South, 58º 13' West; SIRGAS 2000 datum, 216 m) (Fig. 1a – b). A male of *V. ritae* (SVL = 19.8 mm) was photographed, recorded and collected on 6 dec 2017 in the municipality of Sinop, Mato Grosso, Brazil (11º 24' South, 55º 28' West; data datum SIRGAS 2000, 434 m.) (Fig. 1c – d). Another male of *V. ritae* (SVL = 19.9 mm) was collected on 16 feb 2018 at the Sinop Lake hydroelectric plant (11º 24' South, 55º23' West; datum SIRGAS 2000, 428 m). All three specimens were euthanized using a 2 % lidocaine solution and had their liver removed and preserved in 98 % ethanol. Each specimen was subsequently fixed in 10 % formaldehyde, preserved in 70 % ethanol and deposited in the Biological Collection of Southern Amazonia (ABAM) of Universidade Federal do Mato Grosso (UFMT), Campus Sinop, Brazil (ABAM-H 2596, ABAM-H 3085, ABAM-H 3650). The collection permit was issued by Instituto Chico

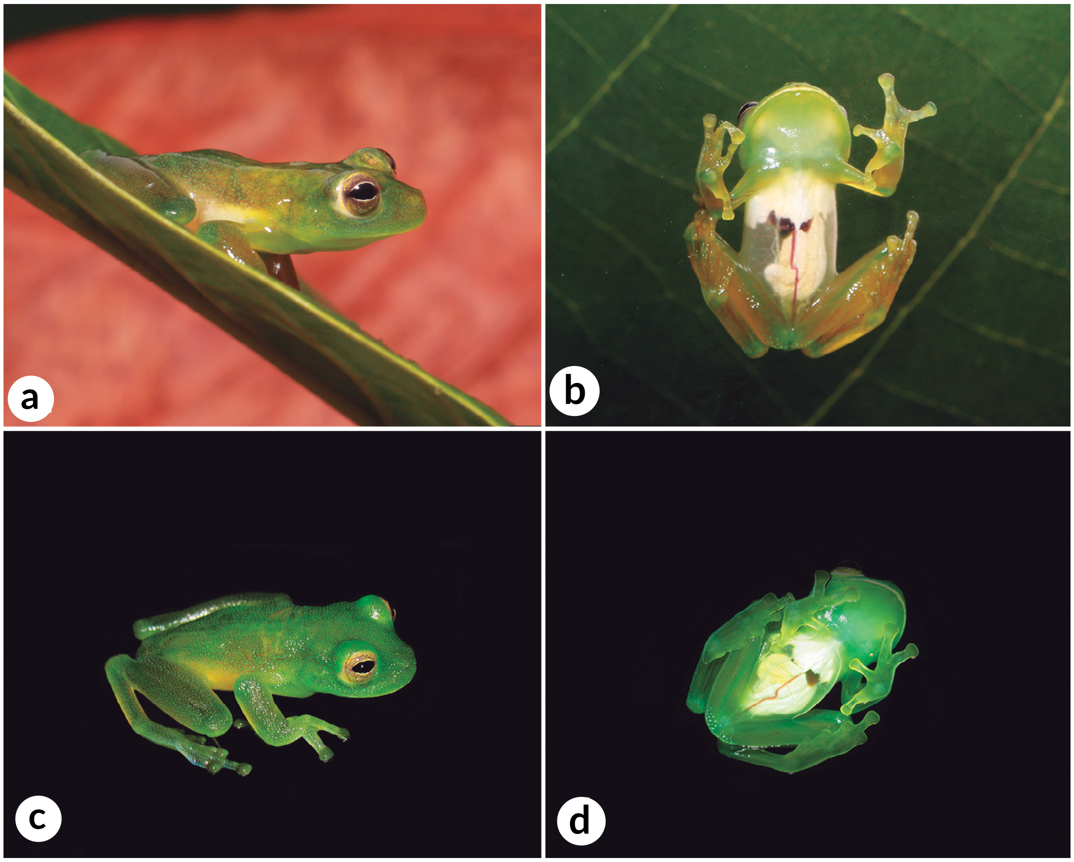

**Figure 1.** Individuals of *Vitreorana ritae* recorded in southern Amazonia. **a-b.** Female, municipality of Cotriguaçu, state of Mato Grosso, Brazil (ABAM-H 2596); **c-d.** Male, municipality of Sinop, state of Mato Grosso, Brazil (ABAM-H 3085). (Photos a, b and d by Marcos Penhacek; photo c by Domingos J. Rodrigues).

Mendes de Conservação da Biodiversidade (SISBIO n º 30034-1).

An egg clutch in the final stages of embryonic development was also found on the same shrub where the male was found in the municipality of Sinop. The clutch was photographed, collected and taken to the UFMT laboratory, Campus Sinop, where it was kept in an aquarium (40 x 60 x 50 cm). After hatching, two tadpoles were euthanized using a 2 % lidocaine solution, fixed in 5 % formaldehyde and deposited in ABAM (ABAM-H 50, ABAM-H 52). The external morphology of the tadpoles was compared with descriptions of other *Vitreorana* tadpoles (e.g. Menin *et al.* 2009). The developmental stage of the tadpoles was determined according Gosner (1960).

Two approaches were used to identify the adult frogs. The first combined morphological data (presence of vomerine teeth; presence and pigmentation of hepatic and intestinal peritoneum; bone and dorsum coloration; and face shape) and bioacoustic data described by several authors (Lutz and Kloss 1952, Lescure 1975, Lescure and Marty 2000, Guayasamin *et al.* 2006, Cisneros-Heredia and Mcdiarmid 2007, Guayasamin *et al.* 2009, Menin *et al.* 2009, Cisneros-Heredia 2013). The second employed BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), to compare DNA sequence data of one of the collected specimens with *V. ritae* sequences found in GenBank.

### Acoustic analysis

Two advertisement calls of a male were recorded using a Sennheiser ME 66 directional microphone coupled to a professional Marantz PMD 660 digital recorder set at a sampling rate of 44.1 kHz and 16-bit resolution and positioned 1.0 m from the specimen. The calls were analyzed using Raven Pro 1.5 (Bioacoustics Research Program c2014). Temporal properties were obtained from oscillograms (temporal resolution of 500 ms), while spectral information was obtained from spectrograms using Fast Fourier Transformation (FFT) with a 256-point window and a frequency resolution of 90 Hz. Seven parameters of the advertisement call were Measured: three temporal — (1) call duration (s), (2) interval between calls (s) and (3) call rate (call / min.); and four spectral — (1) number of harmonics, (2) minimum frequency (5 %) (Hz), (3) dominant frequency (highest peak of energy) including all harmonics (Hz), and (4) maximum frequency (95 %) (Hz). Terminology for the acoustic parameters followed Köhler *et al.* (2017). Sonograms and spectrograms were produced using the R package Seewave (Sueur *et al.* 2008) and tuneR (Ligges *et al.* c2014), with the following settings: FFT size of 256 points, Hanning window, and 90 % overlap. avy

### Molecular analysis

Total genomic DNA was extracted from the liver of the male collected in the municipality of Sinop using the 2 % CTAB protocol (Doyle and Doyle 1990). A 16S mtDNA fragment was amplified through PCR by applying 16Saf and 16Sbr primers (Palumbi *et al.* 1991). The sequencing reaction was performed according to the manufacturer's recommendations for the sequencing mix ABI BigDye Terminator using the primer 16Saf. The sequences (ca. 600 Pb) were aligned using the ClustalW algorithm (Thompson *et al.* 1994) with BioEdit 7.2 software (Hall 1999). The uncorrected pairwise distance (p-distance) between the *Vitreorana ritae* populations of this study and those of GenBank from French Guiana and *V. helenae* from French Guiana and Venezuela were calculated using MEGA 6.0 (Tamura *et al.* 2013).

## RESULTS

The specimens collected were identified as *Vitreorana ritae* based on the vomerine teeth, a white hepatic peritoneum partially covering the liver, a white gastrointestinal peritoneum, green bones in life, dorsal green coloration with dark green spots in life and lavender with dark spots in preservative, and a rounded snout in dorsal view and slightly inclined in lateral view (Fig. 1). The new records of *V. ritae* in the municipalities of Cotriguaçu and Sinop, Mato Grosso (Fig. 2), extends the distribution of the species by 530 and 560 km, to the southeast and south, respectively, from the nearest known distribution of the species (Altamira National Forest, Pará, Brazil; ICMBio c2009), and 1500 and 1700 km, respectively, to the southeast of the type locality (Colombia) (Flores 1987, Cisneros-Heredia 2013).

The egg clutch was found on the adaxial face of a leaf hanging at a height of 1.5 m over a small permanent stream (1.2 m width). The clutch contained 21 embryos wrapped in an off-white, translucent gelatinous capsule (Fig. 3a). The embryos were at stage 21 (Gosner 1960) and exhibited a greenish vitellum and head and a greenish brown tail. After five days in an aquarium, the gelatinous capsule became whitish and opaque (Fig. 3b) and the tadpoles hatched at approximately stage 25. At this stage, the

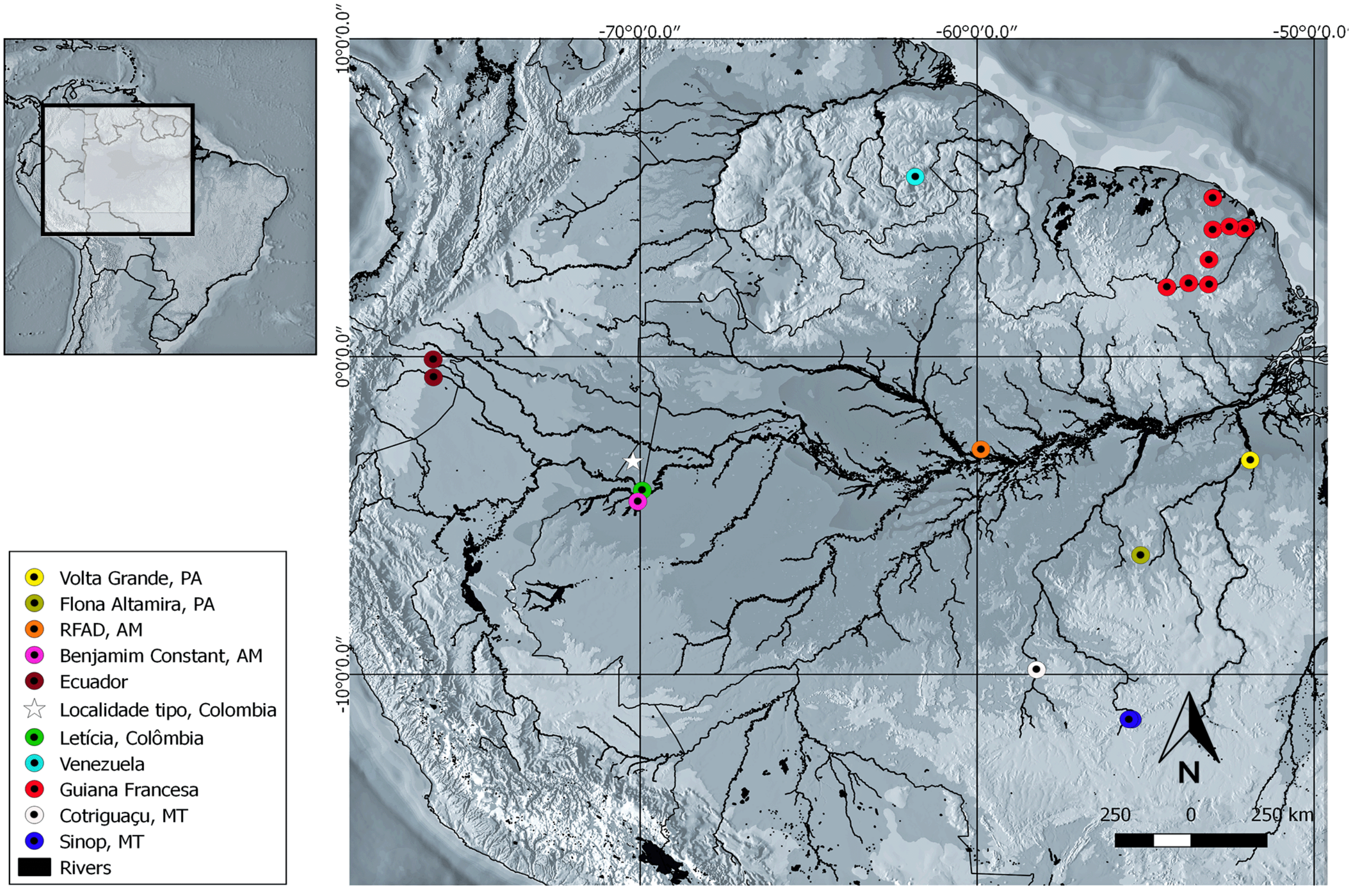

**Figure 2.** Records of *Vitreorana ritae* in the Amazon. Volta Grande, state of Pará, Brazil (Vaz-Silva *et al.* 2015); Flona Altamira, state of Pará, Brazil (ICMBio c2009); RFAD, state of Amazonas, Brazil (Lima *et al.* 2006, Menin *et al.* 2009); Benjamim Constant, state of Amazonas, Brazil (Lutz and Kloss 1952); Ecuador (Ortiz *et al.* c2018); Colombia (Lynch 2005, Cisneros-Heredia 2013); Venezuela (Señaris and Ayarzagüena 2005); and French Guiana (Lescure 1975, Lescure and Marty 2000, GBIF c2017).

tadpoles remained with green vitellum, but with a slightly translucent greenish brown stem and head (Fig. 3c–d). The tadpoles did not develop any further after falling into the aquarium water, at which point they had an elongated and flat body with a subterminal oral disc, a labial tooth row formula (LTRF) of 0/1–2, large marginal papillae and unserrated or arched jaw sheaths.

The calling male was located on the top of a leaf (25 x 15 cm) at a height of approximately 3.5 m over a small stream. The advertisement call consists of a single pulsed note with two visible frequencies (Fig. 4). The duration of the two recorded calls were 0.29 and 0.30 s (N = 2), with a call interval of 1.43 min. The average minimum frequency was 4392 Hz (4306 and 4478 Hz, N = 2), the maximum frequency was 4995 Hz (for both calls), and the average dominant frequency was 4737 Hz (4651 and 4823 Hz, N = 2). The frequency of the second harmonic averaged 9990 Hz (9646 and 10335 Hz, N = 2) (Fig. 4).

Molecular analysis of 16S mtDNA (ABAM-H 3085) from Mato Grosso revealed a 1 % difference with *V. ritae* collected in French Guiana and 4 – 5 % difference with vouchers of its sister species *V. helenae* collected in French Guiana and Venezuela (Table 1).

## DISCUSSION

The new records of *Vitreorana ritae* extend the known distribution of the species 560 km south of the nearest known locality (ICMBio c2009), and thus represents the southernmost known population of this species. These new records, combined with previous records for glass frogs in the Brazilian Amazon (i.e., Noronha *et al.* 2012, Pontes and Mattidi 2013, Oliveira *et al.* 2017, Araújo *et al.* 2018), indicate that there is an insufficient sampling in many regions and that the geographic distribution of most species is not fully known. Furthermore, many of these species lack natural history data (Noronha and Rodrigues 2018).

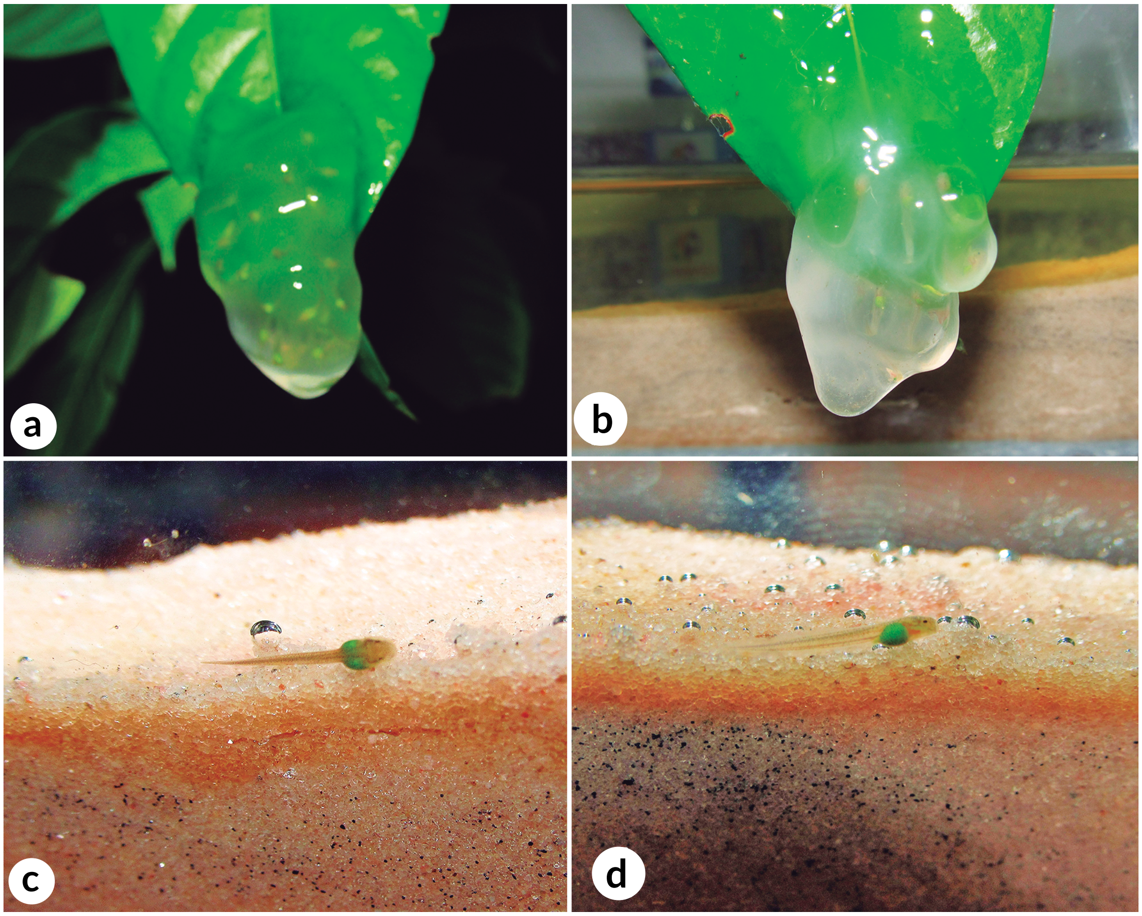

**Figure 3.** Egg clutch and tadpoles of *Vitreorana ritae* collected in southern Amazonia. **a.** Clutch in riparian fores, municipality of Sinop, state of Mato Grosso, Brazil; **b.** same clutch in the laboratory (ABAM); **c.** dorsal and **d**. lateral views of a tadpole at stage 25 (Gosner 1960), (Photos M. Penhacek).

Adult individuals of *V. ritae* are generally difficult to locate and males call sporadically (Zimmerman and Bogart 1984). Reproductive activity of *V. ritae* occurs mainly between January and May, when males can be observed calling from vegetation above streams and where females deposit clutches on upper or lower leaves. Eggs hatch after several days and the embryos fall into the water where they complete their development as free-swimming tadpoles (Menin *et al.* 2009). The streams where *V. ritae* reproduces support strong water currents during the rainy season, and so the tadpoles possess a fossorial habit. This likely explains why few centrolenid tadpoles have been observed and collected throughout the vast region of the Brazilian Amazon. The morphological description of the tadpoles originating from the clutch found in Mato Grosso agrees with that of Menin *et al.* (2009).

The advertisement call of *V. ritae* recorded in southern Amazonia (municipality of Sinop, MT) is a single pulsed harmonic note, which differs from the number of notes of the calls described for the species by Zimmerman and Bogart (1984) for central Amazonia (two notes), Señaris and Ayarzagüena (2005) for Venezuela (two notes), and Lescure and Marty (2000) in Guyana (four notes). Call duration also differed from the calls described from central Amazonia (average 130 ms, Zimmerman and Bogart 1984) and Guyana (average 107 ms, Lescure and Marty 2000). The dominant frequency reported here for southern Amazonia (average 4737 Hz, 4651 Hz and 4823 Hz, N=2) was similar to that reported for males of central Amazonia (4640 – 5160 Hz, Zimmerman and Bogart 1984) and Venezuela (3810 – 4920 Hz, Señaris and Ayarzagüena 2005), but differed from that described for males of Guyana (5570 – 7470 Hz, Lescure and Marty 2000). The calling male reported here exhibited a long period of silence between calls, which was also reported by Zimmerman and Bogart (1984). Although the acoustic parameters of the advertisement calls differ among regions, all these studies analyzed a very small number of individuals and calls, which limits the ability to understand intra- and inter-individual variation and variation among populations. In addition, environmental (e.g., temperature) and social (e.g., number

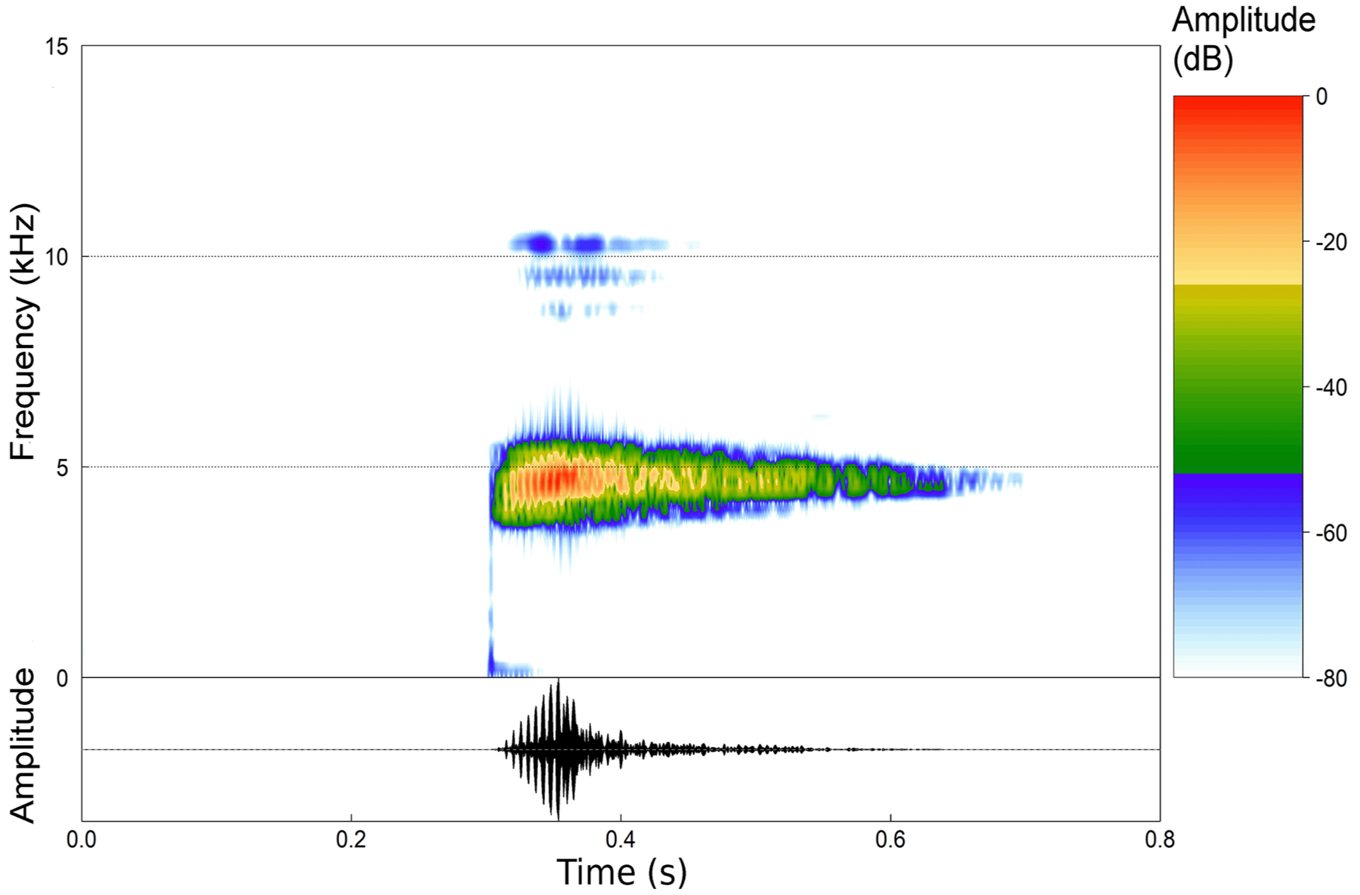

**Figure 4.** Sonogram and spectrogram of the advertisement call of *Vitreorana ritae* in southern Amazonia, Brazil. Voucher (ABAM-H 3085). December 6 2017, 21:55 pm, 27°C, 95% humidity, SVL = 19.8 mm.

of calling males) contexts can influence temporal parameters, such as the number of notes, for example (Morais *et al.* 2012, Köhler *et al.* 2017). Since anurans can have wide vocal repertoires (Toledo *et al.* 2015), it is also possible that the calls described for *V. ritae* represent different types of calls.

Amphibians generally have a low vagility and high philopatry, which are characteristics that promote differentiation and ultimately speciation (Wells 2012, Lima *et al.* 2020). In this sense, although most anuran species would be expected to have small geographic distributions with distantly separated populations having greater genetic differences, there are some cases of widely distributed species with low genetic distances, such as *Dendropsophus nanus* (Boulenger, 1889) (Fouquet *et al.* 2007), *Allophryne ruthveni* Gaige, 1926 (Castroviejo-Fisher *et al.* 2012), and *Adelphobates galactonotus* (Steindachner, 1864) (Rojas *et al.* 2020). There are also cases of low genetic distances between distinct species, such as *Pseudopaludicola jaredi* Andrade, Magalhães, Nunes-de-Almeida, Veiga-Menoncello, Santana, Garda, Loebmann, Recco-Pimentel, Giaretta, and Toledo, 2016 (Andrade *et al.* 2016) and *Proceratophrys ararype* Mângia, Koroiva, Nunes, Roberto, Ávila, Sant'Anna, Santana, and Garda, 2018 (Mângia *et al.* 2018). The low genetic distance among populations of *V. ritae* supports the hypothesis that some anuran species can maintain low genetic distances even though they have wide geographic distributions (Wynn and Heyer 2001, Fouquet *et al.* 2007).

The present work contributes to the knowledge of *V. ritae* and demonstrates that even though it is widely distributed throughout the Amazon and Cerrado transition zone, the genetic, morphological and acoustic characteristics of the species are highly conserved, which may indicate the maintenance of gene flow among populations. The results presented here also demonstrate the capacity of the species has to adapt to different climates and environments by eradicating to lowland forests of the Amazon in drier areas such as the ecotone with the Cerrado. Finally, we emphasize the need for greater sampling in southern Amazonia because the diversity of the region is

**Table 1.** Uncorrected distance (p-distance) between specimens of *Vitreorana ritae* and *V. helenae* from different locations: (1) *V. ritae* from the state of Mato Grosso, Brazil; (2–3) *V. ritae* from French Guiana (EU663017, MB 165 voucher; EU266755, MB 165); (4) *V. helenae* from French Guiana; and (5–8) *V. helenae* from Venezuela.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | |
| **2** | 0.01 | | | | | | | |
| **3** | 0.01 | 0.00 | | | | | | |
| **4** | 0.04 | 0.04 | 0.04 | | | | | |
| **5** | 0.04 | 0.04 | 0.04 | 0.00 | | | | |
| **6** | 0.05 | 0.05 | 0.05 | 0.00 | 0.00 | | | |
| **7** | 0.05 | 0.05 | 0.05 | 0.00 | 0.00 | 0.00 | | |
| **8** | 0.05 | 0.05 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | |

underestimated. Furthermore, the region has been experiencing major environmental impacts, from accelerated expansion of agribusiness and industrial exploitation of natural resources, which may cause the extinction of numerous populations or even endemic species before they are even known scientifically.

## LITERATURE CITED

Andrade FD, Magalhães FDM, Nunes-De-Almeida CHL, Veiga-Menoncello ACP, Santana DJ, Garda AA, Loebmann D, Recco-Pimentel SM, Giaretta AA, Toledo LF. 2016. A new species of long-legged *Pseudopaludicola* from northeastern Brazil (Anura, Leptodactylidae, Leiuperinae). Salamadra. 52(2):107–124.

Araújo KC, Pansonato A, Oliveira RH, Moraisa DH, Carvalho VT, Ávila RW. 2018. Advertisement call and new distribution records from Brazil of *Teratohyla midas* (Lynch and Duellman, 1973) (Anura, Centrolenidae). Check List 14(2):303–308. doi: https://doi.org/10.15560/14.2.303

Bioacoustics Research Program. c2014. RAVEN PRO: interactive sound analysis software, Version 1.5. [last accessed: 04 Nov 2018]. www.birds.cornell.edu/raven

Castroviejo-Fisher S, Vilà C, Ayarzagüena J, Blanc M, Ernst R. 2011. Species diversity of Hyalinobatrachium glassfrogs (Amphibia: Centrolenidae) from the Guiana Shield, with the description of two new species. Zootaxa. 3132(1):1–55. doi: https://doi.org/10.11646/zootaxa.3132.1.1

Castroviejo-Fisher S, Pérez-Pena PE, Padial JM, Guayasamin JM. 2012. A Second Species of the Family Allophrynidae (Amphibia: Anura). Am. Mus. Novit. 3739:1–17. doi: https://doi.org/10.1206/3739.2

Castroviejo-Fisher S, Guayasamin JM, Gonzalez-Voyer A, Vilà C. 2014. Neotropical diversification seen through glassfrogs. J. Biogeogr. 41(1):66–80. doi: https://doi.org/10.1111/jbi.12208

Cisneros-Heredia DF, Mcdiarmid RW. 2007. Revision of the characters of Centrolenidae (Amphibia: Anura: Athesphatanura), with comments on its taxonomy and the description of new taxa of glassfrogs. Zootaxa. 1572(1):3–82. doi: https://doi.org/10.11646/zootaxa.1572.1.1

### *AUTHOR'S CONTRIBUTION*

MP was responsible for collecting data, identifying the specimens, acoustic analysis and writing the text; SFA identified the specimens and revised the text; EAO was responsible for molecular analysis, wroted the text and created the image; EJHR wroted the text; VGB was responsible for acoustic analysis and writing the text; LRRR was responsible for molecular analysis and reviewing the text; DJR was responsible for collecting data, identified the specimens and reviewing the text.

### *CONFLICT OF INTEREST*

The authors declare that they have no conflict of interest.

### *ACKNOWLEDGMENTS*

We thank UFMT for its logistical support. Thanks to FAPEMAT (Proc. No. 300729/2010) and CNPq for financial support (Proc. No. 558225 / 2009-8, 501.408 / 2009-6, 457466 / 2012-0). To IBAMA and Sisbio de Mato Grosso for collection license no. 30034-1. ONF-Brazil and Farm São Nicolau for logistical support. Dr. Luis Reginaldo Ribeiro Rodrigues from UFOPA, Campus of Santarém – Pará, for the genetic analysis of V. ritae (ABAM-H 3085). SFA is a master fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (process nº 88887.199355/2018-00).

Cisneros-Heredia DF. 2013. *Centrolene ritae* Lutz es un sinónimo senior de *Cochranella oyampiensis* Lescure y *Cochranella ametarsia* Flores (Anura: Centrolenidae). Av. Cienc. Ing. 5(2):B1–B4. doi: https://doi.org/10.18272/aci.v5i2.132

Cocroft RB, Ryan MJ. 1995. Patterns of advertisement call evolution in toads and chorus frogs. Anim. Behav. 49(2):283–303. doi: https://doi.org/10.1006/anbe.1995.0043

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus. 12(1):13–15.

Flores G. 1987. A new *Centrolenella* from the Amazonian lowlands of Colombia. J. Herpetol. 21(3):185–190. doi: https://doi.org/10.2307/1564481

Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ. 2007. Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. PLoS ONE 2(10): e1109. doi: https://doi.org/10.1371/journal.pone.0001109

Frost DR. c2019. Amphibian Species of the World. [last accessed: 04 Apr 2019]. http://research.amnh.org/herpetology/amphibia/index.html

[ICMBio] Instituto Chico Mendes de Conservação da Biodiversidade. c2009. Planejamento Estratégico do Mosaico "Terra do Meio". Avaliação Ecológica Rápida. Relatório Técnico – Componente Herpetofauna. [last accessed: 25 Aug 2019]. http://www.icmbio.gov.br/portal/images/stories/docs

Gerhardt HC, Huber F. 2002. Acoustic communication in insects and anurans: common problems and diverse solutions. Chicago: University of Chicago Press.

GBIF Secretariat. c2017. The reptiles and amphibians collection (RA) of the Muséum national d'Histoire Naturelle (MNHN - Paris). [last accessed: 08 Apr 2019]. https://www.gbif.org/dataset/217b0d4f-c87e-4063-b6c5-78277e40e866

Glaw F, Köhler J, De la Riva I, Vieites DR, Vences M. 2010. Integrative taxonomy of Malagasy treefrogs combination of molecular genetics, bioacoustics and morphology Boophis. Zootaxa. 2383(1):82. doi: https://doi.org/10.11646/zootaxa.2383.1.1

Gonçalves ANN. 2013. Influência dos fatores ambientais e da distância espacial na comunidade de anuros de florestas de terra-firme na terra-firme na Amazônia oriental. [Thesis]. [Belem/PA]: Museu Paraense Emilio Goeldi e Universidade Federal do Pará.

Goicoechea, N, De La Riva I, Padial JM. 2010. Recovering phylogenetic signal from frog mating calls. Zool. Scri. 39(2):141–154. doi: https://doi.org/10.1111/j.1463-6409.2009.00413.x

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetol. 16(3):183–190.

Guayasamin JM, Cisneros-Heredia DF, Yánez-Muñoz M, Bustamante M. 2006. Amphibia, Centrolenidae, *Centrolene ilex, Centrolene litorale, Centrolene medemi, Cochranella albomaculata, Cochranella ametarsia:* Range extensions and new country records. Check List. 2(1):70–75. doi: https://doi.org/10.15560/2.1.70

Guayasamin JM. 2008. Taxonomic identity of *Cochranella petersi* Goin, 1961 and *Centrolenella ametarsia* Flores, 1987. Zootaxa. 1815(1):25–34. doi: https://doi.org/10.11646/zootaxa.1815.1.2

Guayasamin JM, Castroviejo-Fisher S, Trueb L, Ayarzagüena J, Rada M, Vilá C. 2009. Phylogenetic systematics of Glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. Zootaxa. 2100(1):1–97. doi: https://doi.org/10.11646/zootaxa.2100.1.1

Guerra VB, Llusia D, Gambale PG, de Morais AR, Márquez R, Bastos RP. 2018. The advertisement calls of Brazilian anurans: historical review, current knowledge and future directions. PLoS One. 13(1): e019169. doi: https://doi.org/10.1371/journal.pone.0191691

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic. Acids. Symp. Ser. 41:95-98.

Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF., Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. Zootaxa. 4251(1):1-124. doi: https://doi.org/10.11646/zootaxa.4251.1.1

Kok PJR, Castroviejo-Fisher S. 2008. Glassfrogs (Anura: Centrolenidae) of Kaiteur National Park, Guyana, with notes on the distribution and taxonomy of some species of the family in the Guiana Shield. Zootaxa. 1680(1):25–53. doi: https://doi.org/10.11646/zootaxa.1680.1.2

Lescure J. 1975. Contribution a l'etude des amphibiens de Guyane Francaise. V Les centrolenidae. Bull. Soci. Zoo. Franc. 100:385–394.

Lescure M, Marty C. 2000. Atlas des Amphibiens de Guyane. Patri. Natu. 45:1–388.

Ligges U, Krey S, Mersmann O, Schnackenberg S. c2014. c/5. TuneR: Analysis of music. [last accessed:20 Oct 2018]. http://r-forge.r-project.org/projects/tuner

Lima AP, Magnusson WE, Menin M, Erdtmann LK, Rodrigues DJ, Keller C, Hödl W. 2006. Guia de sapos da reserva Adolpho Ducke. Manaus: Áttema Design Editorial.

Lima JEP, Margarido VP, Moresco RM, Rodrigues DJ. 2020. Analysis of the mitochondrial D-Loop reveals that neither river boundaries nor geographic distance structure the fine-scale genetic variation of an Amazonian treefrog. Hydrobiologia. 847:321–330. doi: https://doi.org/10.1007/s10750-019-04069-0

Lynch JD. 2005. Discovery of the richest frog fauna in the world – an exploration of the forests to the north of Leticia. Rev. Acad. Colomb. Cienc. Exactas. Fis. Nat. 29(113):581–589

Lutz B, Kloss GR. 1952. Anfíbios anuros do alto Solimoes e Rio Negro, apontamentos sobre algunas formas e suas vicariantes. Mem. Inst. Oswaldo Cruz. 50:625–678. doi: https://doi.org/10.1590/S0074-02761952000100020

Mângia S, Koroiva R, Nunes PMS, Roberto IJ, Ávila RW, Sant'Anna AC, Santana DJ, Garda AA. 2018. A New Species of *Proceratophrys* (Amphibia: Anura: Odontophrynidae) from the Araripe Plateau, Ceará State, Northeastern Brazil. Herpetol. 74(3):255–268. doi: https://doi.org/10.1655/Herpetologica-D-16-00084.1

Menin M, Lima AP, Rodrigues DJ. 2009. The tadpole of *Vitreorana oyampiensis* (Anura, Centrolenidae) in Central Amazonia, Brazil. Zootaxa. 2203(1):65–68. doi: https://doi.org/10.11646/zootaxa.2203.1.6

Moraes JCL, de Almeida AP, de Fraga R, Zamora RR, Pirani RM, Silva AAA, Carvalho VT, Gordo M, Werneck FP. 2017. Integrative overview of the herpetofauna from Serra da Mocidade, a granitic mountain range in northern Brazil. Zookeys. 715:103–159. doi: https://doi.org/10.3897/zookeys.715.20288

Morais AR, Guerra VB, Gambale PG, Signorelli L, Bastos RP. 2012. Acoustic communication in a Neotropical frog (*Dendropsophus minutus*): vocal repertoire, variability and individual discrimination. Herpetol. J. 22(4):249–257.

Noronha JC, Rodrigues DJ, Barros AB, Almeida EJ. 2012. New record and distribution map of *Hyalinobatrachium cappellei* (van Lidth de Jeude 1904) (Anura: Centrolenidae). Herpetol. Notes. 5:467-468.

Noronha JC, Rodrigues DJ. 2018. Reproductive behaviour of the glass frog *Hyalinobatrachium cappellei* (Anura: Centrolenidae) in the Southern Amazon. J. Nat. Hist. 52(3–4):207–224. doi: https://doi.org/10.1080/00222933.2017.1414324

Oliveira EA, Hernández-Ruz EJ. 2017. New species of Glassfrog, Genus *Hyalinobatrachium* (Anura: Centrolenidae), for the Brazilian Amazon Revealed by Mitochondrial DNA and Morphology. Int. J. Res. Stud. Biosci. 5(3):41–52. doi: https://doi.org/10.20431/2349-0365.0503007

Ortiz DA, Guayasamin JM, Frenkel. c2018. Anfibios del Ecuador. Version 2018.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. [last accessed: 31 Oct 2018]. https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Vitreorana%20ritae

Oseen KL, Wassersug RJ. 2002. Environmental factors influencing calling in sympatric anurans. Oecologia. 133(4):616–625. doi: https://doi.org/10.1007/s00442-002-1067-5

Padial JM, Köhler J, Muñoz A, De La Riva I. 2008. Assessing the taxonomic status of tropical frogs through bioacoustics: Geographical variation in the advertisement calls in the *Eleutherodactylus discoidalis* species group (Anura). Zool. J. Linn. Soc. 152(2):353–365. doi: https://doi.org/10.1111/j.1096-3642.2007.00341.x

Palumbi SR., Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. The simple fool's guide to PCR, version 2.0. Honolulu: University of Hawaii.

Pontes RC, Mattedi C. 2013. An unexpected record of *Teratohyla midas* (Lynch and Duellman, 1973) for Brazil reveals the presence of glassfrogs in the Brazilian northern lowlands (Anura: Centrolenidae). Check List. 9(6):1590–1591. doi: https://doi.org/10.15560/9.6.1590

Rojas D, Lima AP, Momigliano P, Simões PI, Dudaniec RY, Avila-Pires TCS, Hoogmoed MS, Bitar YOC, Kaefer IL, Amézquita A, Stow A. 2020. The evolution of polymorphism in the warning coloration of the Amazonian poison frog *Adelphobates galactonotus*. Heredity. 124:439–456 1-18. doi: https://doi.org/10.1038/s41437-019-0281-4

Señaris JC, Ayarzagüena J. 2005. Revisión taxonómica de la familia Centrolenidae (Amphibia; Anura) de Venezuela. Sevilla: Comité español del programa MAB de la UNESCO.

Sueur J, Aubin T, Simonis C. 2008. Seewave, a free modular tool for sound analysis and synthesis. Bioacoustics. 18(2):213–226. doi: https://doi.org/10.1080/09524622.2008.9753600

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30(12):2725–12729. doi: https://doi.org/10.1093/molbev/mst197

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W. improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22(22):4673–4680. doi: https://doi.org/10.1093/nar/22.22.4673

Toledo LF, Araújo OGS, Ávila RW, Kawashita-Ribeiro RA, Morais DH, Cisneros-Heredia DF. 2009. Amphibia, Anura, Centrolenidae, *Cochranella adenocheira*: distribution and range extension, Brazil. Check List. 5(3):380–382. doi: https://doi.org/10.15560/5.3.380

Toledo LF, Martins IA, Bruschi DP, Passos MA, Alexandre C, Haddad CFB. 2015. The anuran calling repertoire in the light of social context. Acta Ethol. 18(2):87–99. doi: https://doi.org/10.1007/s10211-014-0194-4

Vaz-Silva W, Oliveira R, Gonzaga A, Pinto K, Poli F, Bilce T, Penhacek M, Wronski L, Martins J, Junqueira T, Cesca L, Guimarães V, Pinheiro R. 2015. Contributions to the knowledge of amphibians and reptiles from Volta Grande do Xingu, northern Brazil. Braz. J. Biol. 75(3):205–218. doi: https://doi.org/10.1590/1519-6984.00814BM

Venâncio NM, Souza MB, Kokubum MNC. 2014. *Hyalinobatrachium mondolfii* Senari and Ayarzagüena, 2001 (Anura: Centrolenidae): First record for the state of Acre, Brazil. Check List. 10(5):1184–1186. doi: https://doi.org/10.15560/10.5.1184

Zimmerman BL, Bogart JP. 1984. Vocalizations of primary forest frogs species in the central Amazon. Acta Amaz. 14(3-4):473–519. doi: https://doi.org/10.1590/1809-43921984143519

Wells KD. 2012. The Ecology and Behavior of Amphibians. Chicago: University of Chicago Press.

Wynn A, Heyer WR. 2001. Do geographically widespread species of tropical amphibians exist? An estimate of genetic relatedness within the Neotropical frog *Leptodactylus fuscus* (Schneider 1799) (Anura Leptodactylidae). Trop. Zool. 14(2):255–285. doi: https://doi.org/10.1080/03946975.2001.10531157