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New Species of Glassfrog, Genus *Hyalinobatrachium* (Anura: Centrolenidae), for the Brazilian Amazon Revealed by Mitochondrial DNA and Morphology

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Abstract: *This work describes a new species of Hyalinobatrachium for the Brazilian Amazon, in the Tapajós biogeographic region, between the Xingu and Tapajós rivers, two large tributaries of the Amazon River. The new species distinguishes itself from all congeneric species by morphological data and genetic distance based on the 16S mitochondrial gene fragment. The new species differentiates from its congeneric ones mainly by the absence of the nuptial excrescence, the white disc I finger and the peritoneum of the yellow gallbladder. Geneticaly for H. munozorum, H. fleischmanni and H. carlesvilai and morphologically similar to H. mondolfii. It will be the sixth one registered for Brazil, occurring in the Tapajós biogeographic region.*

Keyword: *Amphibia, Xingu, Hyalinobatrachium mondolfii, Hyalinobatrachium munozorum, Hyalinobatrachium muiraquitan* ***sp. nov.***

Abbreviations: *snout–vent length (SVL); head length (HL); head width (HW); interorbital distance (IOD); eye length (EL); upper eyelid width (EW); eye to snout tip distance (ES); width of disc on Finger III (DW); femur length (FEL); tibia length (TL); foot length (FL).*

1. INTRODUCTION

Currently 30 species of *Hyalinobatrachyum* Ruiz-Carranza and Lynch 1991, are described [1], distributed in tropical Central America, tropical Andes, the Cordillera of the Coast of Venezuela, Tobago, the Amazon Basin and the Shield of the Guianas [2]. Only five of these are registered for Brazil [3]: *Hyalinobatrachium carlesvilai* Castroviejo-Fisher, Padial, Chaparro, Aguayo-Vedia and De la Riva, 2009, *Hyalinobatrachium cappellei* Van Lidth de Jeude, 1904, *Hyalinobatrachium iaspidiense* (Ayarzagüena, 1992), *Hyalinobatrachium mondolfii* Señaris and Ayarzagüena, 2001, and *Hyalinonatrachium munozorum* (Lynch and Duellman, 1973). In the state of Pará *H. iaspidiense* [1] and *H. mondolfii* [4] were recorded.

The taxonomy of glass frogs, as they are popularly known, is still quite confusing [5; 6; 7; 8], making it difficult to exactly assess the diversity of species and their distributions, even with the taxonomy works carried out in the Ecuador [9], Venezuela [10], Guiana Shield [8] and Guiana [11]. Due to the morphological similarities found within the genus [10; 12], these species pose a problem for conservation measures, because distinct species may be masked because of phenotypic conservation, presenting a restricted distribution. A solution to this taxonomic problem may be applying integrative taxonomy, which takes into account several lines of evidence and criteria to identify divergent lineages [13; 14; 15; 16; 17], assuming that species are segments of lineages of separately evolving metapopulations [18; 19; 20; 21; 22].

Based on morphological and molecular data, we describe a new species of *Hyalinobatrachyum* for Brazil, morphologically similar to *H. mondolfii*. It will be the sixth one registered for the country, occurring in the Tapajós biogeographic region, which is considered an area of important endemism for conservation and studies on the origin of the Amazonian biota [23; 24]. The new species differentiates from its congeneric ones mainly by the absence of the nuptial excrescence, the white disc I finger and the peritoneum of the yellow gallbladder.

2. MATERIAL AND METHODS

2.1. Nomenclature

This study is based on literature data and analyzes of specimens collected in the field with ecological data information. The specimens used in this study were fixed in 10% formalin and preserved in 70% alcohol. The specimens and data examined are listed in Additional Information 2. The characters quoted here are for adults only. Ontogenetic status was determined by the presence of secondary sexual characters (vocal sac and nuptial callus), as well as field data (vocalization). Two couples were collected in amplexo, with posterior spawning in plastic bags. The credit for the photos goes to Leandro de Souza Mello. We follow [2; 25] for the supra-specific classification. The specimens were collected with the authorization for collection and transportation 32401 from SISBIO, Brazil.

2.2. Morphology

For terminology and definitions of morphological characters and coloration, we follow [2; 3; 5; 6; 8; 10;]. The terminology of basal networks follows [26]. Description of the nuptial excrement (bridal callus) and prepolical thorn follows [27], with additions of [2]. Coloring characteristics were observed in living individuals, color photographs and literature data [2; 6; 10; 13].

The species described in this work distinguishes itself from all other species mainly in comparisons of qualitative morphological characters and molecular synapomorphies. Due to the difficulty in accessing materials stored in zoological collections, we have studied and compared the characters listed and described in detail by [8; 28] for the species of *Hyalinobatrachium,* drawing comparisons with the new species.

The morphometric measurements were made with the help of digital caliper 0.01mm. Literature data from the holotype of *Hyalinobatrachium mondolfii* [28] was used. Abbreviations for measurements are as follows: snout–vent length (internarial tip of the snout to the posterior edge of cloacal opening, SVL); head length (posterior edge of the jaw articulation to the internarial tip of the snout, HL); head width (greatest width of head, at the posterior edge of the jaw articulation, HW); interorbital distance (distance between the inner margins of the orbits, IOD); eye length (horizontal diameter, EL); upper eyelid width (greatest transverse width, EW); eye to snout tip distance (from tip of snout to anterior margin of eye, ES); width of disc on Finger III (DW); femur length (distance from the middle of the cloacal slit to the femur-tibia articulation, FEL); tibia length (from the femur-tibia to the tibia-heel articulation, TL); foot length (distance from proximal margin of outer metatarsal tubercle to tip of Toe IV, FL).

We consider that a taxon presented divergence in morphology when displaying at least one fixed (qualitative) character, no overlap (quantitative) or a single combination separating this from other taxa, according to [8]. The purpose of this assumption is that fixed differences in morphology are a strong indication of reduced or absent gene flow [29], evidence of independent lineages.

2.3. Molecular Analysis

Total genomic DNA was extracted from two specimens of *Hyalinobatrachium* aff. *mondolfii* of Brazil. Additionally, sequences from *H. mondolfii* available in the GenBank were employed, as well as other species of the genus (Table 1) using the pheneno-chloroform protocol [30]. A fragment of the 16S DNAr gene was amplified via PCR using the *primers* 16Saf and 16Sbr [31]. Amplification was performed under the following conditions: 60s at 92°C followed by 35 cycles of 92°C (60 s), 50°C (50 s) and 72°C (1.5 min). The final volume of the PCR reaction was 12 µL and it contained 4.4 µL of ddH_2O, 1.5 µL of 25 mM $MgCl_2$, 1.25 µL of 10 mM dNTPs (2.5mM each dNTP), 1.25 µL of buffer 10x (75 mM Tris HCl, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), 1 µL of each *primer* (2 µM), 0.3 µL of 1 U Taq DNA Polymerase and 1 µL of DNA (30 – 50 ng/µL).

The sequencing reaction was performed according to the manufacturer's recommendations for the sequencing mix ABI *BigDye Terminator*, using the 16Saf primer at an annealing temperature of 50°C. The sequencing reactions were precipitated using the standard protocol of EDTA/Ethanol, resuspended with 10µL of deionized formamide (ABI) and sequenced on the automatic sequencer ABI 3130xl (*Applied Biosystems*).

The sequences were aligned using the Clustal W algorithm [32] Implemented in BioEdit software 7.2 [33]. The evolutionary molecular model GTR + G was chosen through the software Jmodel Test [34]. The tree Maximum Likelihood was built in software *Treefinder* [35] with 20,000 replicates of *bootstrap*. The genetic distance pairwise uncorrected (*p*-distance) among species was calculated in software MEGA 6.0 [36].

Table1. *List of specimens of Hyalinobatraachium used in molecular analysis*

Species	Nº GenBank	Locality	Status
H. muiraquitan **sp. nov.**	KY310570	Vitória do Xingu	Voucher
H. muiraquitan **sp. nov.**	KY310571	Vitória do Xingu	Voucher
H. mondolfii	JF266569.1	Venezuela	Voucher
H. mondolfii	JN870870.1	Guiana	Voucher
H. mondolfii	GQ1420461	Venezuela	Voucher
H. mondolfii	EU6630501	Venezuela	Voucher
H. fleischmanni	KR863261.1	Panamá	Voucher
H. cf. *munozorum*	EU663034	-------------	------------
H. duranti	EU663041.1	-------------	Voucher
H. ibama	EU663048.1	-------------	Voucher
H. orientale	EU447289.1	Venezuela	Voucher
H. orocostale	EU447288.1	Venezuela	voucher
H. fragile	EU663046.1	-------------	voucher
H. carlesvilai	KM068269.1	-------------	voucher
H. carlesvilai	KM068270.1	-------------	voucher
H. fleischmanni	KR863261.1	Panamá	voucher
H. bergeri	GQ142059.1	-------------	voucher
H. talamancae	FJ784480.1	Panamá	voucher
Hyalinobatrachium sp.	KM068299.1	-------------	voucher
H. pellucidum	GQ142065.1	-------------	voucher
H. colymbiphyllum	KR863254.1	Panamá	voucher
H. chirripoi	KF604299.1	Panamá	voucher
H. aureoguttatum	EU663032.1	-------------	voucher
H. valerioi	EU663057.1	-------------	voucher
H. taylori	JN870873.1	Venezuela	voucher
H. crurifasciatum	EU663040.1	-------------	voucher
H. eccentricum	EU663042.1	-------------	voucher
H. ignioculus	EU663049.1	-------------	voucher
H. pallidum	EU663052.1	-------------	voucher
H. iaspidiense	JN870867.1	Brazil	voucher
Cochranella revocata	EU663019.1	-------------	--------------

Bayesian Analysis of Population Structure (BAPS) was implemented in the software BAPS 5.0 [37] to find clusters formed with the sequences obtained in this study and available in GenBank. The BAPS software uses nucleotide frequencies of the samples to infer the K number of different groups genetically by Bayesian analysis, allocating similar sequences in the same group. The maximum number of K chosen for the analysis was three, corresponding to the number of locations (Brazil, Guyana and Venezuela). The log-likelihood value of the best model was used to choose the most likely clustering configuration.

3. RESULTS AND DISCUSSION

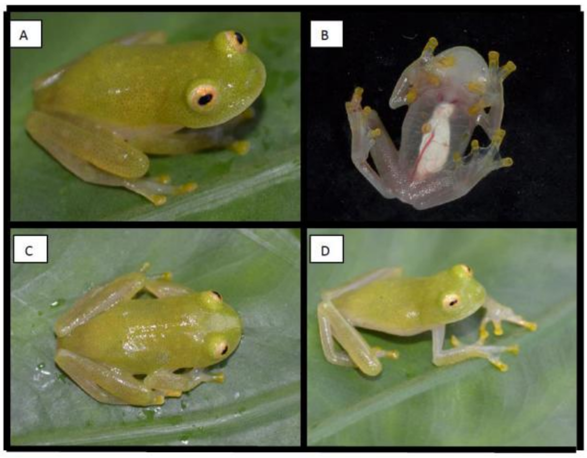

Figure1. *Hyalinobatrachium muiraquitan* **sp. nov**. *In life collected at the Vitória farm in the municipality of Vitória do Xingu, Pará - Brazil. Dorsal view in A, C and D, ventral view in B. (photo: Leandro Souza de Melo).*

3.1. Holotype (LZA 841)

Adult male collected January 15, 2016 by Elciomar Araújo de Oliveira and Joyce Celerino de Carvalho at Vitoria farm (S: 02º58"00' and W 052º13"00) municipality of Vitória do Xingu, State of Pará, Brazil.

3.2. Paratypes

One adult male (LZA 842) and two adult females (LZA 843 and LZA 844) collected in the same locality of the holotype.

3.3. Genus *Hyalinobatrachium*

The new species belongs to the genus *Hyalinobatrachium*, as evidenced by sharing the states of the characters indicated in, [38; 39; 5]: (1) absence of humeral spines; (2) digestive tract and liver bulbs covered by white peritoneum; (3) ventral parietal peritoneum completely transparent; (4) white bones in life, green in *H. mesai* Barrio-Amorós and Brewer-Carias, 2008 and *H. taylori* (Goin, 1968); (5) dorsal coloration white or cream when in alcohol; (6) males without dorsal spines in reproductive age; (7) when present, the nuptial callus is small and restricted to the inner side of finger I of the hand of the males; (8) dentigerous process of vomer and absent vomiting teeth; (9) males usually vocalize on the underside of the leaves, and the females lay a layer of eggs in the same location; and (11) complete fusion of the tibia and fibula.

3.4. Diagnosis

(1) dentigerous process of vomer and absent vomerine teeth; (2) face rounded in dorsal view and truncated in side view; (3) tympanus covered by skin (4) dorsally, smooth skin or weakly shagreen in life and alcohol; (5) ventrally, granular or smooth skin, absent warts on the sewer; (6) pericardium and white hepatic peritoneum, all other peritones are transparent, yellow gallbladder peritoneum; (7) bulbous liver; (8) absent humeral spine; (9) formula of the membranes of the fingers III 2 $(0^- – 0^-)$ IV; (10) basal network on toes I (1 – 1) – $(1_{3/4} – 1_{1/4})$ II $(1_{1/4} – 1)$ – $(2 – 1_{1/2})$ III (2-0) - (3 – 0) IV (3 – 0) – $(2 – 0^-)$ V; (11) absent nuptial excrescences, pre-polex not evident in external view; (12) finger I longer than finger II; (13) diameter of the eye thinner than the disc of hand finger III; (14) coloring in life: yellow dotted dorsum forming light green and tiny black melanophores extending through the limbs, white bones; (15) coloring in alcohol: light cream dotted with tiny black melanophores; (16) gilded iris with gray halftones near the pupil; (17) tiny black melanophores present only at the base of the IV finger and at the base of the IV and V toes. In life the discs of the fingers are all yellow, while in the feet, the disk of toe I is white, while all the others are yellow; (18) deposits of eggs on the underside of the leaves; (19) adult size between 19.2 - 19.6 mm in two males and 20.0-21.8 mm in two females; (20) nineteen molecular autapomorphs on the mitochondrial gene fragment 16S (table 2).

Table2. *Diagnostic characters on the 16S gene fragment for Hyalinobatrachium muiraquitan sp. nov., H. mondolfii and H. munozorum.*

Position	*H. muiraquitan* **sp. nov.**	*H. mondolfii*	*H. munozorum*
20	C	A	A
89	G	A	A
108	G	A	A
127	C	T	T
174	C	T	T
216	A	C	C
227	T	A	A
232	T	T	C
238	T	C	C
247	A	C	C
249	C	T	T
250	C	T	T
253	T	C	C
359	A	G	G
328	C	A	A
331	A	T	T
358	C	A	A
377	C	T	T
492	T	C	C

3.5. Comparisons

The comparison of the new species with the other congeneric species found in the eastern Brazilian Amazon or *fleischmanni* group *sensu* [38; 39] supports their identification based on morphology (characters of the new species in parentheses): *H. mondolfii* has rounded snout in lateral view (truncated), present excrescence nuptial (absent), yellow discs in all feet (white I finger disc), melanophores present only in the V foot (IV and V of the foot) and white gallbladder peritoneum (yellow); *H. munozorum* has a white gallbladder (yellow), absent melanophors (present), basal network present between fingers I and II (absent), rostrum rounded in side view (truncated), absent tarsal and ulnar fold (present); *H. ruedai* Ruiz-Carranza and Lynch, 1998 has formula basal networks of hands III 2 – $1_{1/4}$ IV (*H. muiraquitan*. **sp. nov.** III 2 (0^- – 0) IV), absent melanophors (present); *H. cappellei* has black dots scattered over the back (tiny yellow dots), white discs in the fingers and toes (yellow); *H. iaspidiense* has irregular green spots on its back in life and small black spots (light green with yellow dots and tiny black melanophores); *H. orientale* (Rivero, 1968) for having red heart in life and transparent pericardium (heart and white pericardium). *H. fleischmani* Ruiz-Carranza and Lynch, 1991 has subacuminado rostrum in dorsal view (rounded); *H. ibama* Ruiz-Carranza and Lynch, 1998 has weakly granular dorsal skin texture (dorsal skin texture smooth or weakly shagreen); *H. esmeralda* Ruiz-Carranza and Lynch, 1998 has a formula of the membranes of the fingers III 2^+-2^+ IV (the new species has formula III 2 (0^- – 0^-) IV)

3.6. Molecular Analysis

The population structure analysis performed by BAPS, with the species *Hyalinobatrachium mondolfii* and *H. muiraquitan* **sp. nov**. revealed a strong structuring between the populations of Brazil and Venezuela + Guyana (logML = -409.7148; probability =100%, Figure 2). Based on this result and considering the phylogenetic analysis of the nominal species *Hyalinobatrachium mondolfii*, we observed the existence of two lineages (Figure 3). The first is composed of individuals from the Shield of Guyana and Venezuela, based on GenBank sequences. The second is composed of individuals collected in this study in the city of Vitória do Xingu, state of Pará - Brazil. The two lineages present a genetic distance of 5% and a bootstrap value of 52 supporting the branch of the new species. It can be observed that the genetic distance value is higher than the one currently used to distinguish species of anurans (> 3%), a value that has been strongly defended by several authors based on a 16S mitochondrial gene fragment [40; 41; 42; 43], while species recognized as *H. munozorum* and *H. mondolfii* present only 2% (table 3).

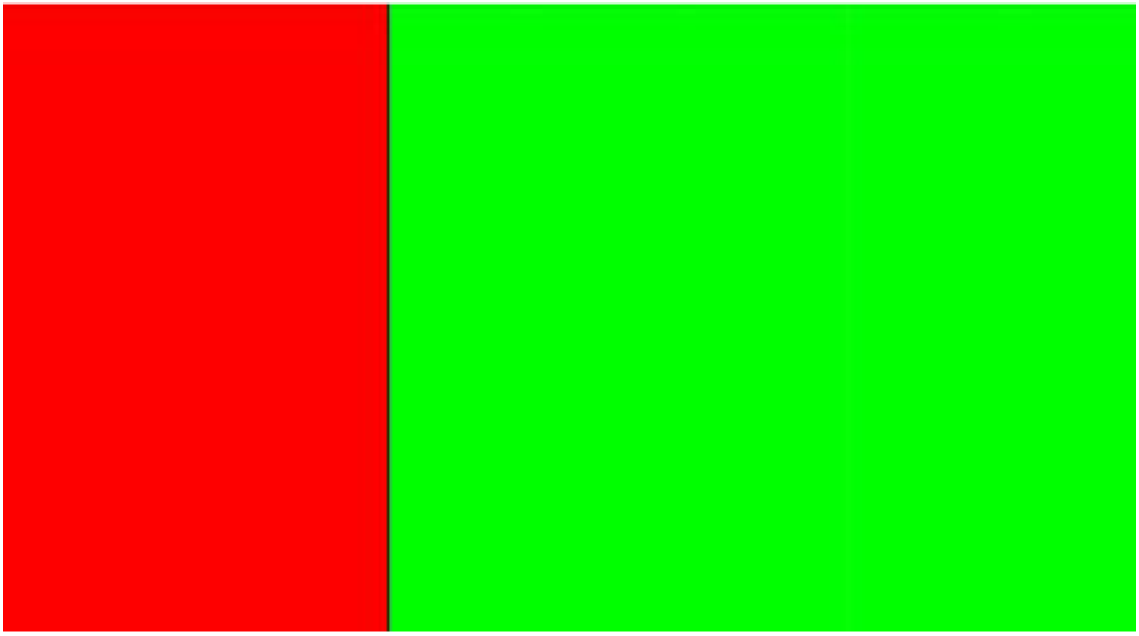

Figure2. *Population structure analysis in BAPS. The colors represent the populations of the Xingu (red) and Venezuela + Guyana (green).*

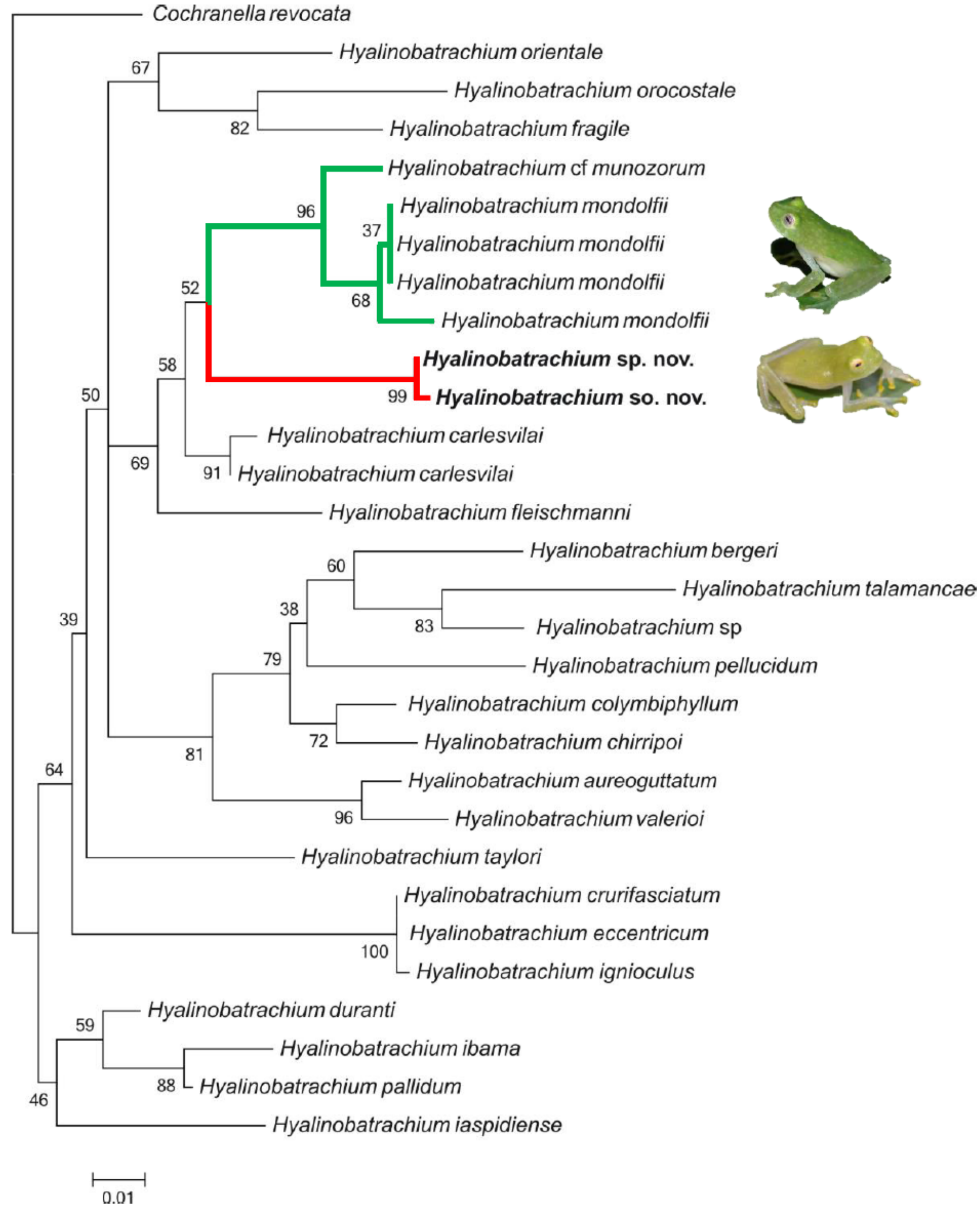

Figure3. *Maximum likelihood tree based on 16S mitochondrial marker. The numbers on the nodes represent the values of boostrtap. The colors in the branches represent the biological grouping found by Bayesian analysis in the software BAPS, Xingu (red) and Venezuela + Guiana (green).*

Table3. *Genetic distance matrix uncorrected (%) between species of Hyalinobatrachium*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1																									
2	0,04																								
3	0,04	0,04																							
4	0,04	0,03	0,03																						
5	0,05	0,02	0,04	0,03																					
6	0,06	0,07	0,06	0,05	0,06																				
7	0,05	0,03	0,04	0,03	0,04	0,05																			
8	0,05	0,05	0,04	0,04	0,05	0,06	0,04																		
9	0,06	0,04	0,05	0,04	0,04	0,05	0,02	0,04																	
10	0,06	0,05	0,05	0,04	0,05	0,06	0,04	0,04	0,05																
11	0,06	0,05	0,05	0,05	0,05	0,06	0,04	0,05	0,05	0,03															
12	0,06	0,06	0,05	0,05	0,05	0,06	0,05	0,06	0,06	0,06	0,06														
13	0,06	0,05	0,05	0,05	0,05	0,06	0,05	0,06	0,06	0,06	0,06	0,00													
14	0,06	0,04	0,04	0,04	0,04	0,06	0,02	0,04	0,01	0,05	0,05	0,06	0,05												
15	0,06	0,05	0,05	0,05	0,05	0,06	0,05	0,06	0,06	0,06	0,06	0,00	0,00	0,05											
16	0,06	0,05	0,06	0,04	0,06	0,06	0,03	0,06	0,04	0,06	0,05	0,06	0,06	0,05	0,06										
17	0,06	0,04	0,05	0,04	0,05	0,07	0,03	0,04	0,04	0,05	0,05	0,06	0,05	0,04	0,05	0,05									
18	0,06	0,05	0,05	0,05	0,05	0,03	0,04	0,05	0,04	0,05	0,05	0,06	0,05	0,05	0,05	0,05	0,05								
19	0,06	0,06	0,06	0,05	0,05	0,04	0,05	0,06	0,05	0,06	0,06	0,05	0,05	0,05	0,05	0,06	0,06	0,04							

20	0,06	0,06	0,06	0,06	0,06	0,05	0,05	0,06	0,05	0,06	0,07	0,07	0,06	0,06	0,06	0,06	0,06	0,04	0,04						
21	0,06	0,05	0,05	0,05	0,06	0,05	0,05	0,05	0,05	0,06	0,05	0,06	0,06	0,05	0,06	0,06	0,05	0,04	0,06	0,05					
22	0,06	0,06	0,06	0,06	0,06	0,05	0,05	0,05	0,05	0,06	0,05	0,07	0,07	0,05	0,07	0,07	0,05	0,04	0,06	0,05	0,02				
23	0,07	0,06	0,05	0,05	0,06	0,04	0,04	0,05	0,04	0,06	0,05	0,06	0,06	0,04	0,06	0,05	0,05	0,02	0,05	0,04	0,04	0,05			
24	0,08	0,07	0,07	0,06	0,07	0,03	0,06	0,06	0,05	0,07	0,05	0,07	0,07	0,06	0,07	0,07	0,07	0,05	0,04	0,06	0,06	0,06	0,05		
25	0,05	0,05	0,05	0,04	0,05	0,05	0,03	0,05	0,04	0,05	0,05	0,05	0,05	0,04	0,05	0,04	0,05	0,04	0,05	0,05	0,05	0,06	0,05	0,06	

1) Hyalinobatrachium muiraquitan sp. nov. (Brasil); 2) H. munozorum; 3) H. fleischmanni; 4) H. carlesvilai; 5) H. mondolfii (Venezuela e Guiana); 6) Hyalinobatrachium sp.; 7) H.duranti; 8) H. orientale; 9) H. pallidum; e 10) H. fragile; 11) H. orocastale; 12) H. ignioculus; 13) H. eccentricum; 14) H. ibama; 15) H. crurifasciatum; 16) H. iaspidiense; 17) H. taylori; 18) H. colymbiphyllum; 19) H. bergeri; 20) H. pellucidum; 21) H. aureoguttatum; 22) H. valerioi; 23) H. chirripoi; 24) H. talamancae and 25) Craugastor revocata.

3.7. Description of Holotype

Adult male with SVL 19.2 mm; HW 39.5 % of SVL; head wider than long (HW/HL = 1.2); rostrum rounded in dorsal view and truncated in side view; ES/EL = 1.6 and ES/IOD = 2; concave loreal region; prominent oval nostrils; depression of internarial region; *canthus rostralis* not evident; small eye (EL 29 % of HL); undistinguished tympanic annulus, absent tympanic membrane, absent tympanic above fold, dentigerous process absent vomer; elongated and ovoid tongue, not fixed to the posterior or side of the mouth, anterior region 2/3 the length of the tongue fixed to the mouth; slits on the side of the base of the tongue; external vocal sac and medium. Lean forearm, without pleats; absent humeral spine; relative length of the fingers: II < I < IV < III; wide discs of hand fingers, only the disc of finger III is truncated; FIII 55 % of EL; basal network absent between fingers I-II and weak basal webbing between fingers II-III and formula of membranes of hand fingers III 2 (0^- – 0^-); subarticular rounded slightly prominent tubercle; rounded and prominent palmar tubercle, elongated and slightly evident thenar tubercle; nuptial excrescence absent on finger and fringes of fingers II, III and IV; slender hind limbs; TL 51,5 % of SVL; rounded toe discs I, II and II and truncated IV and V; small and oval internal metatarsal tubercle; almost absent external metatarsal tubercle; basal toe network I (1 – 1) – ($1_{3/4}$ – $1_{1/4}$) II ($1_{1/4}$ – 1) – (2 – $1_{1/2}$) III (2-0) - (3 – 0) IV (3 – 0) – (2 – 0^-) V. In alcohol, dorsal skin weakly shagreen, area near to the tympanum almost granular; texture of the belly smooth and granular laterally and smooth thighs; cloacal region with few folds close to the opening, absent warts.rounded tubercle and some prominent; rounded and prominent palmar tubercle, thenar.

3.8. Coloring in Life

Light green dorsum with yellow dots; tiny black melanophores extending through the limbs; white bones.

3.9. Coloring in Alcohol

Light cream, dotted with tiny black melanophores.

3.10. Variation

Males have smaller SVL than females (average = 29 mm and 31.8 mm, respectively). Interorbital distance is smaller in males, with an average of 2.3 mm, while in females it is 2.65 mm. Although they were smaller, males presented a larger length of the head than the females (average = 12.7 mm and 9.7 mm respectively). All measurements are in Additional Information 1.

3.11. Etymology

The specific epithet "muiraquitan" refers to artifacts carved in stone (jade, greenish color) or wood, representing people or animals (frog, fish, turtle, etc.), to which the supernatural qualities of the amulet are attributed. This epithet is used for the species due to its similarity with the muiraquitan of the legends, usually represented by greenish-colored frogs.

3.12. Natural History

The two adult males and females were found on the margin of a stream with a strong current, in amplexus. Several males were vocalizing at a height of 40 cm from the soil. Spawns were found at the site, about 11 eggs in a nest of gelatin on the underside of the leaves, on top of the water.

3.13. Distribution

Currently the new species can be found in the municipalities of Vitória do Xingu (locality type) and near the city of Altamira, both in the state of Pará (Figure 4).

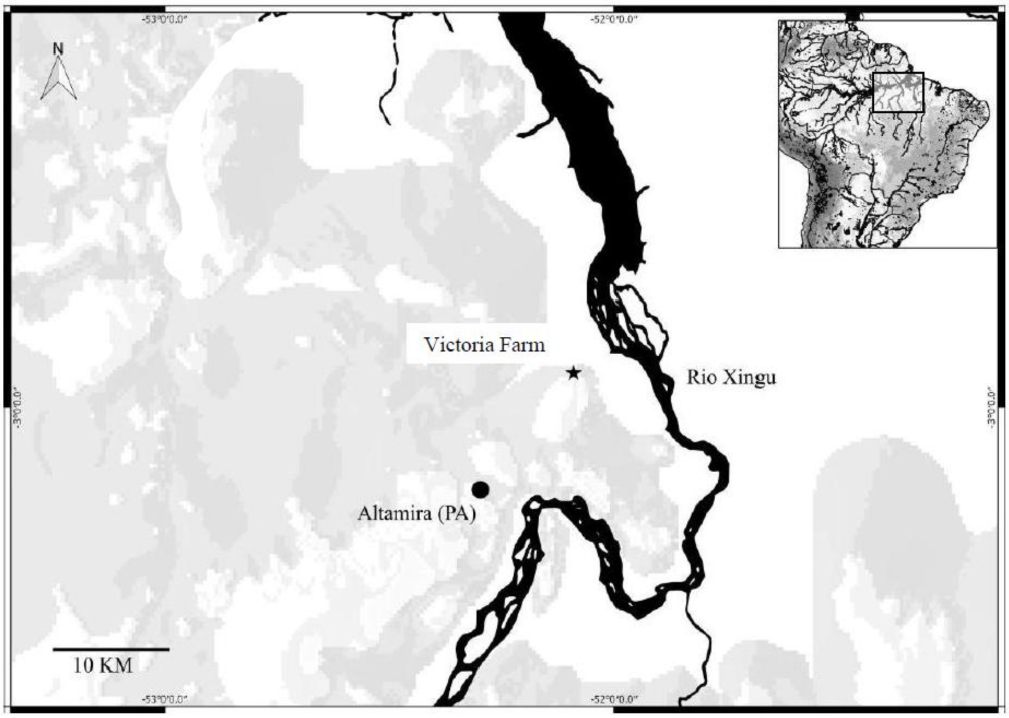

Figure4. *Known distribution map for the new species. The star represents the type locality (Vitória do Xingu) and the circle another point of record of the species (Altamira).*

Cocrhanella sp. was registered in the north of the state of Pará, in the region of the Trombetas River [44]. However, [8] recognizes this record as the species *Hyalinobatrachium mondolfii*, also registered in the Xingu region [4]. After thorough morphological and molecular analyzes, the species registered in Xingu is described as a different one. Due to morphological similarities between anurans, many species are masked under a same name, the so called cryptic species [45]. The difficulty in the correct identification of species by taxonomy poses problems for the actions taken towards species conservation.

Even with the absence of vocalization, the new species is supported by the morphological differences presented above, as well as by molecular data. Despite the low support value separating the new species from its sister species (*H. mondolfii* and *H. munuzorum*), *Hyalinobatrachium muiraquitan* presented a genetic distance from 5% and 4% respectively. Low resolution values in phylogenetic trees were found in *Hyalinobatrachium* [8], *Pristimantis* [46; 47], *Lysapsus* and *Pseudis* [48]. These results indicate that the inclusion of new genes is necessary for a better understanding of the phylogenetic relationships between species.

Hyalinobatrachium muiraquitan has been mistakenly called *H. mondolfii* due to their morphological similarities and the lack of specialists in the group in the Xingu region. Knowing that this is a region of the Amazon with great biological diversity, composing one of the eight areas of endemism [24], taxonomy studies should take priority. Knowledge of the biota of a region helps in making decisions regarding biodiversity conservation and, as such, taxonomy studies [10; 39; 49], phylogenies [8; 50; 46] and inventories [11; 39; 44] become relevant. Amphibians have been studied for some time as bioindicators of environment quality [51; 52]. It is well known that the species that inhabit a place and how they interact with the environment becomes relevant to preserve the biota.

The greatest diversity, with the largest number of species of the genus *Hyalinobatrachium*, is registered for Guiana shield, which includes countries such as Venezuela, Suriname, Guyana, French Guiana, Northern Brazil and a small portion of eastern Colombia [8; 10; 11]. The region presents a great chain of mountains called tepuis, with an altitudinal variation of 400 – 1200 m above sea level [53; 54]. These heterogeneous environmental conditions allowed the emergence of new species and endemism [11].

On the other hand, in the Amazon Basin the high richness of other taxa species such as birds has been associated with a process of specificity in-situ [55], repeated immigration into the Amazon from outside sources, and low levels of extinction within the basin [56]. The major Amazonian rivers often formed barriers to geographically replacing taxa, with closely related species or subspecies occupying opposing river banks [57; 58]. In this sense, *Hyalinobatrachium muiraquitan* can be endemic of the Xingu / Tapajós interfluvium, formed by two great tributaries of the Amazon River. The role of large Amazonian rivers as barriers to anuran species is well documented in the literature [59; 60; 61; 62; 63]. Thus, due to this geographical feature of the Amazon basin, the great rivers play a major role in the diversification of the biota by preventing gene flow between populations of opposite margins, leading to the emergence of new species [24; 64; 65].

4. CONCLUSION

We conclude that *Hyalinobatrachium muiraquitan* can be easily differentiated from the species *H. mondolfii* by the truncated snout present in lateral view, the absence of nuptial excrescence and I finger disc white foot and peritoneum of the yellow gallbladder; from *Hyalinobatrachium munozorum* by present tarsal and ulnar fold, absence of basal webbing between the fingers I and II; from *H. ruedai* by the presence of melanophores in the body; from *H. cappellei* by its small yellow spots scattered around the body and discs of the fingers and yellow toes; from *H. iaspidiense* by its tiny yellow dots and black melanophores in the body.

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ADDITIONAL INFORMATION 1

Measurements of all specimens of *Hyalinobatrachium muiraquitan* **sp. nov.** used in this study.

Indivíduo	Sexo	SVL	HL	HW	IOD	EL	EW	ES	FIII	FEL	TL	FL
LZA 841	M	19.2	8.8	7.6	1.5	1.8	0.9	3.0	3.5	10.3	9.9	8.0
LZA 844	F	21.8	6.4	7.6	1.7	2.1	1.5	2.9	3.4	11.2	11.2	9.1
LZA 843	F	20.0	6.6	8.1	1.9	2.4	1.7	3.0	3.7	10.8	10.6	8.9
LZA 842	M	19.6	6.2	7.5	1.6	2.4	1.8	3.0	3.2	10.7	10	8.7

ADDITIONAL INFORMATION 2

List of specimens used for qualitative morphology based on the literature works cited in this paper.

Hyalinobatrachium mondolfii, Estado Delta Amacuro, Venezuela, MHNLS 12710;

Hyalinobatrachium ruedai, Miraflores, Colômbia, ICN 40409;

Hyalinobatrachium iaspidiense, EBD 28803 (holotype);

Hyalinobatrachium munozorum, KU 155497;

Hyalinobatrachium cappellei, RMNH 37850;

Hyalinobatrachium orientale, Monagas, Cueva del Guácharo, Venezuela, MHNLS 13353; Río Pajaral, afluente del Río Caripe, MHNLS 14750-14752, MHNLS 13354.

Hyalinobatrachium fleischmanni, San Jose, Provincia, Costa Rica, SMF 3760, (holotype);

Hyalinobatrachium ibama, Gambita, Colômbia, ICN 12602;

Hyalinobatrachium esmeralda, Pajarito, Colômbia, ICN 9598.