Herpetology Notes, Volume 18: 861–866 (published online on 15 September 2025)

First record of *Paratelmatobius segallai* Santos et al., 2019 in the state of São Paulo, southeastern Brazil, based on molecular and morphological evidence

Sandro P. de Faria[1,2,3,*], Diego J. Santana[4], Thiago T. Tognolo[5], and Sarah Mângia[4]

Leptodactylid frogs are a highly diverse group of amphibians, ranging from southern Texas, in North America, to South America, encompassing Brazil, Chile, and Argentina (Frost, 2025). Currently, the family comprises 238 species (Pyron and Wiens, 2011; Streicher et al., 2018; Frost, 2025), which are allocated in three subfamilies: Leiuperinae Bonaparte, 1850, Leptodactylinae Werner, 1896 (1838), and Paratelmatobiinae Ohler & Dubois, 2012 (Pyron and Wiens, 2011). Among them, Paratelmatobiinae is the least diverse, consisting of 16 species restricted to the Brazilian Atlantic Rainforest (Fouquet et al., 2013).

Four genera are currently recognised within Paratelmatobiinae: *Crossodactylodes* Cochran, 1938, *Paratelmatobius* Lutz & Carvalho, 1958, *Rupirana* Heyer, 1999, and *Scythrophrys* Lynch, 1971 (Fouquet et al., 2013). Among these, the Atlantic Forest endemic *Paratelmatobius* comprises seven species, which occur across the Serra do Mar and Serra da Mantiqueira mountain ranges (Pombal and Haddad, 1999; Garcia et al., 2009). Species of *Paratelmatobius* are considered rare due to their habitat specificity, short breeding season, and restricted geographic range (Giaretta and Castanho, 1990; Santos et al., 2020). Some species have not been recorded since their original description and remain known only from their type series (e.g., *Paratelmatobius lutzii* Lutz & Carvalho, 1958), while others have gone undetected for several decades (e.g., *Paratelmatobius mantiqueira* Pombal & Haddad, 1999) (Santos et al., 2019).

All species of *Paratelmatobius* share a bright and contrasting ventral colouration and are allocated into two species groups: *P. cardosoi* and *P. lutzii*. The *P. cardosoi* group is characterised by an unflattened head, the presence of vocal slits in males, a first finger longer than the second, and unfringed and unwebbed margin of the first toe, and an undivided nuptial pad in males, and comprises *P. cardosoi* Pombal & Haddad, 1999, *P. mantiqueira*, and *Paratelmatobius yepiranga* Garcia et al., 2009 (Pombal and Haddad, 1999; Garcia et al., 2009; Santos et al., 2019). In contrast, the *P. lutzii* group is distinguished by a flattened head, the absence of vocal slits in males, a first finger as long as or shorter than the second, a fringed or webbed medial margin of the first toe, and a nuptial pad divided into two portions, and comprises *Paratelmatobius gaigeae* (Cochran, 1938), *P. lutzii*, and *Paratelmatobius poecilogaster* Giaretta & Castanho, 1990 (Pombal and Haddad, 1999; Garcia et al., 2009; Santos et al., 2019). These groupings are further supported by recent multi-locus phylogenetic analyses (Santos et al., 2019, 2020).

Paratelmatobius segallai Santos et al., 2019 was described based on a comprehensive series of individuals from Parque Estadual Pico do Marumbi, municipality of Piraquara, located in the Serra do Mar mountain range, state of Paraná, southern Brazil. This species was assigned to the *P. cardosoi* group based on morphological traits and phylogenetic analysis of mitochondrial DNA fragments, later corroborated by multi-locus phylogenetic analyses (Santos et al., 2019, 2020). Despite its well-documented type series, *P.*

[1] Centro de Pesquisas Itaqui, Estrada Doutor Yojiro Takaoka 4601, Itapevi, São Paulo 06696-050, Brazil.
[2] Centro de Pesquisas e Monitoramento Ambiental da Mata Atlântica - Instituto Terraluminous (CPMAMA-ITL), Estrada da Fazenda Ribeirão Grande 7300, Juquitiba, São Paulo, 06950-000, Brazil.
[3] Carbono Florestal, Rua 1 de maio nº 8, Santo André, São Paulo 09015-030, Brazil.
[4] Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul 79070-900, Brazil.
[5] Sapo Flecha Estudos Ambientais, Rua Ary Camargo Machado 33, Mirassol, São Paulo 15135-644, Brazil.
[*] Corresponding author. E-mail: sandro.faria@carbonoflorestal.com

© 2025 by Herpetology Notes. Open Access by CC BY-NC-ND 4.0.

segallai remains known only from its type locality in Paraná. Although similar *Paratelmatobius* specimens have been recorded elsewhere (e.g., Santos et al., 2020), their taxonomic affinities remain uncertain due to the lack of unambiguous diagnostic characters of external morphology among populations. In this study, we report the first record of *P. segallai* from the state of São Paulo, also in the Serra do Mar mountain range, supported by genetic data and morphological diagnostic characters proposed for the species.

Materials and Methods

Study site and sampling. During fieldwork conducted in the municipality of Pedro de Toledo, state of São Paulo (-24.2750ºS, -47.2380ºW, elevation 702 m; WGS 84), we (SPF and TTT) recorded two individuals of the *Paratelmatobius* genus. The first individual (ZUFMS-AMP19004, Fig. 1) was encountered on 01 March 2021, actively moving over leaf litter at 05:45 h. The second (unvouchered) was encountered on 02 December 2021, buried under leaf litter at 20:00 h. The region is characterised by hills, table-shaped ridges with stepped levels, extensive lowlands, and discontinuous, elongated river terraces. Both specimens were found within 20 meters of each other, in areas with abundant leaf litter. We manually captured and euthanised one specimen (ZUFMS-AMP19004) using 5% lidocaine. A piece of thigh muscle was taken from the specimen prior to fixation and preserved in 95% ethanol for subsequent molecular analyses. The individual was fixed in 10% formalin for 48 hours, washed with water, and transferred to 70% ethanol for permanent storage (following ethical guidelines established by Conselho Federal de Biologia-CFBio; Nº 526/2019, 2012). The voucher specimen was deposited in the zoological collection of Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP), Campo Grande, Brazil. We state here that appropriate protocols for the collection and handling of the individuals were followed for the present research according to Brazilian federal law. The collecting permit was issued by ICMBio (SISBio 45889-1).

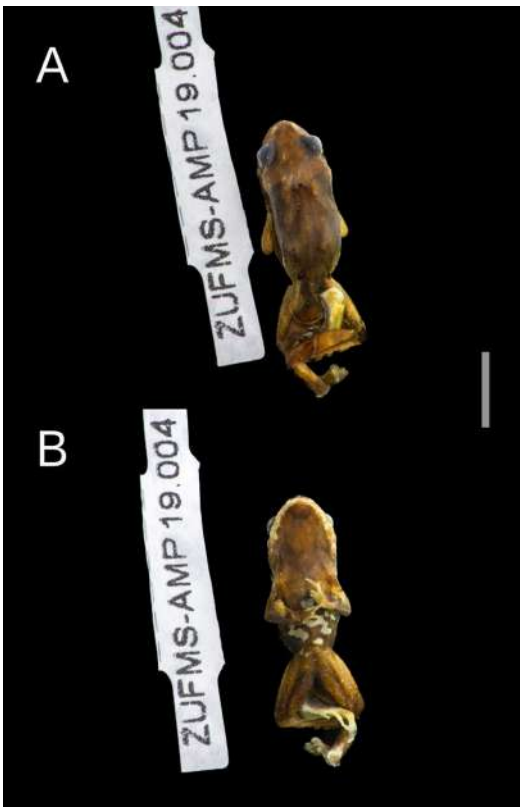

Figure 1. Overview of new specimen of *Paratelmatobius segallai* (ZUFMS-AMP19004) from the municipality of Pedro de Toledo, state of São Paulo, Brazil. A) Dorsal view; B) Ventral view. Scale bar = 10 mm.

Molecular analyses. To verify the phylogenetic relationships of the Pedro de Toledo individuals, we extracted whole genomic DNA from an ethanol-preserved sample of the specimen (ZUFMS-AMP19004), using a phenol-chloroform extraction protocol (Sambrook et al., 1989). We targeted the 16S ribosomal RNA mitochondrial gene, employing primers 16Sa (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16Sb (5′-CCG GTC TGA ACT CAG ATC ACG T-3′) (Palumbi et al., 1991). We configured the PCR protocol with one initial phase of 94 ºC for three minutes, followed by 35 cycles of 94 ºC for 20 s, 50 ºC for 20 s, and 72 ºC for 40 s, with a final extension phase of 72 ºC for five minutes. Purification of PCR products and DNA sequencing were performed by Eurofins Genomics Inc. (Louisville, KY, USA). We edited the chromatograms in Geneious v.7.1.0 software (Kearse et al., 2012).

We combined our new 16S sequence (GenBank accession: PX251554) with all other *Paratelmatobius* sequences that were identified at a species level and available in GenBank. Additionally, we used one Paratelmatobiinae taxa, *Scythrophrys sawayae* (Cochran, 1953), as an outgroup. We aligned sequences using the built-in MAFFT v.7 plugin (Katoh and Toh, 2008) in Geneious v.7.1.0, using default settings. The

resulting alignment consists of 82 terminals and 512 base-pairs (bp) for the 16S rRNA gene fragment. The alignment is available at Github (https://github.com/Rhinella85/Paratelmatobius-segallai-SP); sequences are identified by the specific name, followed by the museum voucher and GenBank accession number.

We conducted a maximum likelihood (ML) phylogenetic analysis using RAxML-HPC (Randomized Axelerated Maximum Likelihood; Stamatakis, 2014), employing a GTR + GAMMA model of nucleotide substitution, which accounts for rate heterogeneity across sites. A rapid bootstrap analysis with 100 replicates (-N 100) was performed alongside a best-scoring maximum likelihood tree search (-f a) to assess branch support. We also calculated uncorrected p-distances for selected *Paratelmatobius* samples to assess genetic distances between the new individual and other congeners, using MEGA v.7 (Kumar et al., 2018). We selected nine individuals (Table 1) from different clades within the genus that were assessed for base substitutions per site from between sequences.

Results

Our ML phylogenetic inference (LnL = –1866.661682) based on the 16S rRNA gene fragment places the newly identified individual (ZUFMS-AMP19004) from the municipality of Pedro de Toledo nested in a clade with *Paratelmatobius segallai* (bootstrap support = 100), including its type series (Fig. 2). Additionally, *P. segallai* is recovered as a monophyletic species, sister to *P. cardosoi* (bootstrap support = 81). Our uncorrected p-distances reveal low distances between our new individual and the holotype of *P. segallai* (CFBH 43545; 1.3% distance, 11 bp difference, Table 1), with moderate distances for other species (3.8% from *P. cardosoi* [CFBH 17679]; 6.8% from *P. gaigeae* [UFMG 15006]; 5.4% from *P. poecilogaster* [MNRJ 75864]; and 5.7% from *P. yepiranga* [MZUSP 136698]).

Both Pedro de Toledo individuals also exhibited morphological concordance with the type series of *P. segallai*, sharing key traits such as small body size (snout-vent-length, SVL = 17.48 mm; SVL type series = 18.6 ± 1.0 [males], 20.6 ± 0.8 [females]), well-developed fringes on the toes, an orange-blotched belly and ventral surfaces of the arms and forearms, the absence of orange blotches on the external margins of the throat, unexpanded toe tips, and a well-developed dorsolateral fold.

Table 1. Pairwise uncorrected p-distances, as inferred in MEGA software, for selected individuals of *Paratelmatobius*. Holotype of *P. segallai* is indicated with a cross (†), and the new record is indicated in bold. Terminals are identified by specific epithet, followed by voucher, and GenBank accession number.

	P_segallai_ZUFMS_BRA_SP_Pedro_Toledo	P_segallai_MK791309.1 (†)	P_cardosoi_MN917490.1	P_cardosoi_MN917507.1	P_gaigeae_MN917539.1	P_gaigeae_MN917543.1	P_poecilogaster_MN917560.1	P_poecilogaster_MN917559.1	P_yepiranga_MN917591.1
P_segallai_ZUFMS_BRA_SP_Pedro_Toledo	-	-	-	-	-	-	-	-	-
P_segallai_MK791309.1 (†)	0.013	-	-	-	-	-	-	-	-
P_cardosoi_MN917490.1	0.038	0.033	-	-	-	-	-	-	-
P_cardosoi_MN917507.1	0.047	0.042	0.015	-	-	-	-	-	-
P_gaigeae_MN917539.1	0.068	0.068	0.063	0.066	-	-	-	-	-
P_gaigeae_MN917543.1	0.068	0.068	0.063	0.066	0	-	-	-	-
P_poecilogaster_MN917560.1	0.054	0.057	0.05	0.056	0.033	0.033	-	-	-
P_poecilogaster_MN917559.1	0.057	0.057	0.053	0.056	0.033	0.033	0.004	-	-
P_yepiranga_MN917591.1	0.057	0.055	0.056	0.067	0.071	0.071	0.059	0.063	-

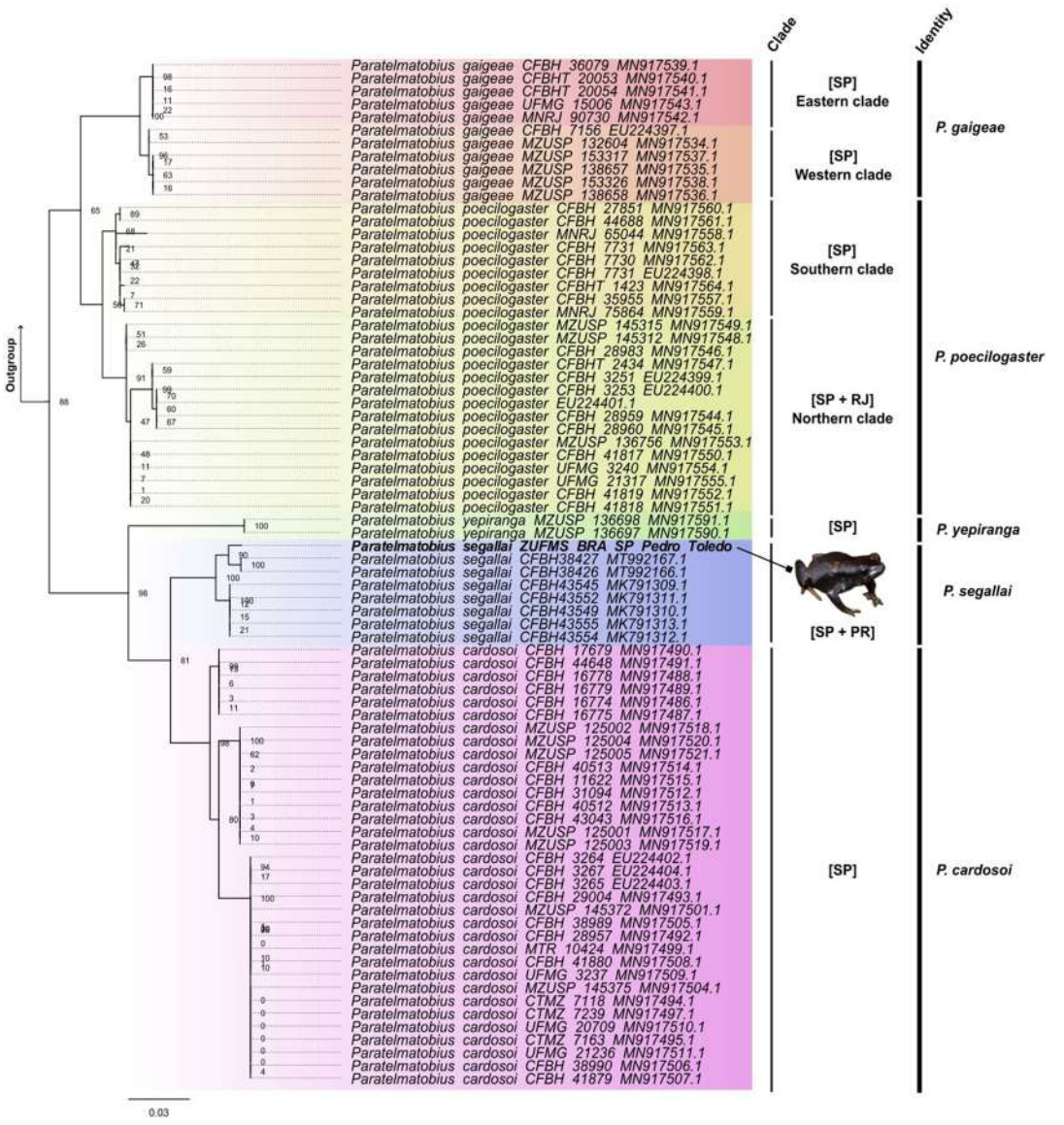

Figure 2. Maximum likelihood phylogenetic inference over the 16S rRNA gene fragment alignment for *Paratelmatobius* spp. and outgroup (*Schytophrys sawayae*). Terminals are identified by specific epithet, followed by voucher, and GenBank accession number. Node numbers refer to bootstrap support values. The new sequence generated in this work is highlighted in bold, followed by inset photograph of the same individual (new record of *P. segallai*, ZUFMS-AMP19004, from municipality of Pedro de Toledo, state of São Paulo, Brazil).

Discussion

Based on the integration of morphological evidence, low genetic distances, and the monophyly of the new individuals with the *P. segallai* type series, we report the first record of *P. segallai* outside of its type locality. This marks its first occurrence of *P. segallai* in the state of São Paulo extending its known geographic range by 223 km north, from the municipality of Piraquara, state of Paraná, to municipality of Pedro de Toledo, state of São Paulo (Fig. 3). Despite this substantially range extension, *P. segallai* remains restricted to the Serra do Mar mountain range. This supports previous suggestions in the literature that *Paratelmatobius* species may

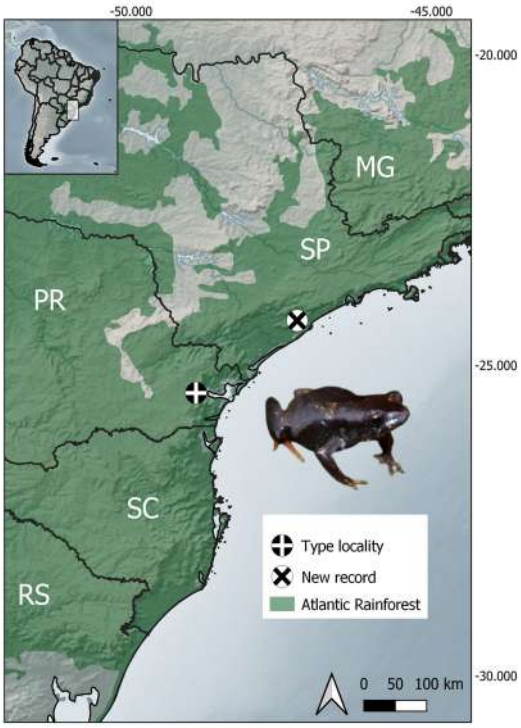

Figure 3. Updated geographic range of *P. segallai*. Type locality in Parque Estadual Pico do Marumbi, municipality of Piraquara, state of Paraná (cross symbol), and new record in municipality of Pedro de Toledo, state of São Paulo (X symbol). Green shading refers to the limits of the Atlantic Rainforest biome. Inset photograph: new individual of *P. segallai* (ZUFMS-AMP19004). State abbreviations: MG = Minas Gerais; PR = Paraná; RS = Rio Grande do Sul; SP = São Paulo. Photo by Sarah Mângia.

be habitat specialists with restricted ranges (Giaretta and Castanho, 1990; Santos et al., 2019). Moreover, the municipality of Pedro de Toledo is located less than 20 km from the São Paulo Metropolitan region, a highly urbanised area comprising 39 municipalities and over 20 million inhabitants (IBGE, 2023). Given this proximity, the few suitable habitats for *P. segallai* may already be under threat from deforestation and habitat degradation. Further faunal inventories, ideally integrating morphological and genetic data, are essential for identifying and documenting new *Paratelmatobius* populations, which will be crucial for informing conservation strategies for these rare species and their habitats.

References

Fouquet, A., Blotto, B.L., Maronna, M.M., Verdade, V.K., Juncá, F.A., De Sá, R., Rodrigues, M.T. (2013): Unexpected phylogenetic positions of the genera *Rupirana* and *Crossodactylodes* reveal insights into the biogeography and reproductive evolution of leptodactylid frogs. Molecular Phylogenetics and Evolution **67**(2): 445–457.

Frost, D.R. (2025): Amphibian Species of the World: an online reference. Version 6.2. Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. Accessed on 09 January 2025.

Garcia, P.C.D.A., Berneck, B.V.M., Da Costa, C.O.R. (2009): A new species of *Paratelmatobius* (Amphibia, Anura, Leptodactylidae) from Atlantic rain forest of southeastern Brazil. South American Journal of Herpetology **4**(3): 217–224.

Giaretta, A.A., Castanho, L.M. (1990): Nova espécie de *Paratelmatobius* (Amphibia, Anura, Leptodactylidae) da Serra do Mar, Brasil. Papéis Avulsos de Zoologia **37**: 133–139.

IBGE [Instituto Brasileiro de Geografia e Estatística] (2023): Censo Demográfico 2022: população e domicílios: primeiros resultados. Coordenação Técnica do Censo Demográfico. Rio de Janeiro, IBGE.

Katoh, K., Toh, H. (2008): Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics **9**(4): 286–298.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Drummond, A. (2012): Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**(12): 1647–1649.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018): MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution **35**(6): 1547–1549.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991): The simple fool's guide to PCR, version 2.0. Vol. 45. Honolulu: University of Hawaii, 26–28.

Pombal Jr, J.P., Haddad, C.F. (1999): Frogs of the genus *Paratelmatobius* (Anura: Leptodactylidae) with descriptions of two new species. Copeia **1999**(4): 1014–1026.

Pyron, R.A., Wiens, J.J. (2011): A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution **61**(2): 543–583.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular cloning: a laboratory manual. 1546 pp. Cold Spring Harbor Laboratory Press, NY.

Santos, M.T.T., Oliveira, S.H., Carvalho, T.R., Zaidan, B.F., Silva, N.R., Berneck, B.V.M., Garcia, P.C.A. (2019): A new species of *Paratelmatobius* (Anura: Leptodactylidae: Paratelmatobiinae) from the Atlantic Forest of southern Brazil. Zootaxa **4648**(3): 473–493.

Santos, M.T.T., de Magalhaes, R.F., Lyra, M.L., Santos, F.R., Zaher, H., Giasson, L.O., Haddad, C.F. (2020): Multilocus phylogeny of *Paratelmatobiinae* (Anura: Leptodactylidae)

reveals strong spatial structure and previously unknown diversity in the Atlantic Forest hotspot. Molecular Phylogenetics and Evolution **148**(106819): 1–18.

Stamatakis, A. (2014): RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics **30**(9): 1312–1313.

Streicher, J.W., Miller, E.C., Guerrero, P.C., Correa, C., Ortiz, J.C., Crawford, A.J., Wiens, J.J. (2018): Evaluating methods for phylogenomic analyses, and a new phylogeny for a major frog clade (Hyloidea) based on 2214 loci. Molecular Phylogenetics and Evolution **119**: 128–143.

Accepted by Fábio Hepp