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Short Communication

Rediscovery of the critically endangered annual killifish *Leptopanchax itanhaensis* (Costa, 2008) in a temporary pool and roadside ditch from the Atlantic Forest of Southeast Brazil

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Abstract: We report new records of the critically endangered *Leptopanchax itanhaensis* in a temporary pool near its type locality and in a roadside ditch from the same sub-basin. Despite extensive collection efforts over the years to find the species and concerns of potential regional extinction, five individuals were successfully sampled in February and March 2024. However, those newfound records raise concerns regarding the urgent necessity for conservation efforts in the species' habitat. The current area, where the species was rediscovered, is under imminent threat of deforestation, emphasizing the imperative for ongoing monitoring.
Keywords: *Temporary environments; Rivulidae; Itanhaém; Rio Preto; conservation.*

Redescoberta do killifish anual criticamente ameaçado *Leptopanchax itanhaensis* (Costa, 2008) em uma poça temporária e vala de estrada da Mata Atlântica do Sudeste Brasileiro

Resumo: Relatamos um novo registro do criticamente ameaçado *Leptopanchax itanhaensis*, em uma poça temporária próxima à localidade-tipo e em uma vala de estrada da mesma sub-bacia. Apesar dos extensos esforços de coleta ao longo dos anos para encontrar a espécie e das preocupações com uma potencial extinção regional, cinco indivíduos foram amostrados com sucesso em Fevereiro e Março de 2024. No entanto, estes novos registros levantam o alerta em relação à urgente necessidade de esforços de conservação do habitat da espécie. A área atual, onde a espécie foi redescoberta, está sob ameaça iminente de desmatamento, enfatizando a necessidade de monitoramento contínuo.
Palavras-chave: *Ambientes temporários; Rivulidae; Itanhaém; Rio Preto; conservação.*

Introduction

There is a substantial gap in our understanding of the distribution of species inhabiting the temporary habitats within the Atlantic Forest, owing to the challenging geomorphological landscape features of this region (Costa 2002, Menezes et al. 2007). Moreover, the Atlantic Forest houses a significant proportion (36%) of Brazil's threatened small freshwater fish species and, within this context, the Rivulidae constitutes over 40% of the country's endangered small freshwater fish species (Castro and Polaz 2020). Numerous species, including those within the family, are on the verge of extinction, raising concerns that some may remain undiscovered (Bohlke et al. 1978, Volcan and Lanés 2018).

Leptopanchax is a genus of small fish, up to 5 cm (2.0 in) long, in the family Rivulidae, which most members are rarely documented (Costa 2019) and face imminent threats of extinction or are possibly already extinct (Costa 2008). Recent decades have witnessed the rediscovery of other rivulid species (Costa 1998, 2013, Costa et al. 2019, Guedes et al. 2023). Similarly, additional species from the same genus previously considered extinct, were reported after 31 years (Costa 2013) and 74 years (Costa et al. 2019) of their last known records. The prolonged hiatus in recording these species is a direct consequence of the extensive deforestation of their habitats, particularly in lowland moist forests, posing considerable challenges to the conservation priorities of killifish residing in these ecosystems (Costa 2019).

Presently, *Leptopanchax itanhaensis* (Costa, 2008), formerly described as *Leptolebias itanhaensis*, a small Rivulidae that lives in dark acidic waters and shallow pools nestled in dense moist forest habitats in the coastal plain (Costa 1995, 2008, 2019), is considered as Critically Endangered (CR, Pavanelli et al. 2018). Few information on the biology of the species is available, however, it is known that there are difficulties in reproduction in captivity, with subpopulations not surviving more than one generation, and that diving into the substrate to spawn is optional, with the couple being able to lie down on the bottom of the puddle to spawn (Costa 2008). The eggs of the species have a spherical shape, and a diameter between 896 and 1005 µm, with a reticulate surface, with reticulum forming irregular pentagons (or hexagons) with greatest width of 3.4–6.5% egg diameter, and with mushroom-like projections distributed regularly on the egg surface (Costa and Leal 2009). Until now, the species was only recorded in its type locality, in the Itanhaém River basin, southeastern Brazil (Costa 2008, Pavanelli et al. 2018). Unfortunately, the pool where the species was originally sampled and described was destroyed in 2007, and despite the intensive collection efforts reported, the species, until now, had not been recorded again in the area, prompting concerns that it might have become extinct (Pavanelli et al. 2018). Against this backdrop, our findings confirm the rediscovery of *L. itanhaensis* in the Itanhaém region of southeastern Brazil, marking a significant finding after many years of limited information about its occurrence. In this short communication we also describe some structural and physicochemical variables of one temporary pool and one roadside ditch where five individuals of *L. itanhensis* were recently collected, in addition to the species occurrence in these two habitats.

Materials and Methods

The individuals were sampled in a temporary pool (24°13'17.4"S 46°55'37.2"W) and in a roadside ditch created for road water drainage (24°11'18.6"S 46°54'30.2"W), on the coastal plain of the Atlantic Forest, drained by the Rio Preto sub-basin, Rio Itanhaém coastal basin, Itanhaém, southeastern Brazil (Figure 1), in February and March 2024. Fish were captured using hand nets, and since the specimens were injured due to sampling, the species were anesthetized and euthanized with 0.5g/L of Tricaine methanesulfonate (MS-222) neutralized with 1.0 g/L NaHCO3, and kept in absolute alcohol, with the aim of preserving them for future analyses of their physiology, genetics, trophic ecology and fecundity, in addition to their deposit in a collection. Subsequently, they were identified following Costa (2008, 2019); morphometric measurements were taken using calipers following Costa (1995) and Guedes et al. (2020), and individuals were also weighed on an analytical balance. Afterward, they were deposited in the UNESP Fish Collection (DZSJRP 24845 and 24846). All sampling and procedures were carried out under the SISBIO 90241-1 license and CEUA – IB/CLP nº 15/2023. At the sampling sites, physicochemical parameters (pH, temperature (°C), dissolved oxygen (mg/L), turbidity (NTU), and conductivity (mS/cm)), were assessed using a Horiba U-52 G multiprobe. Additionally, the maximum length, maximum width, and maximum depth of the pool and roadside ditch were measured in meters.

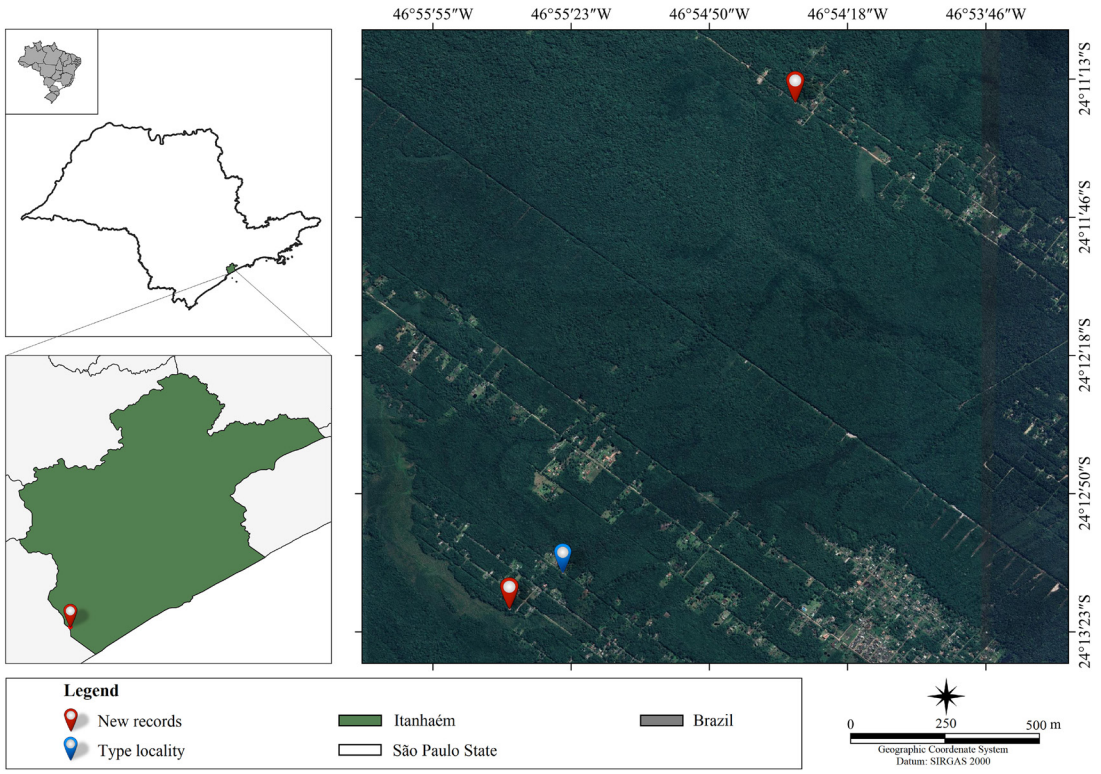

Figure 1. New records of *Leptopanchax itanhaensis* collected at a temporary pool (24°13'17.4"S 46°55'37.2"W) and roadside ditch (24°11'18.6"S 46°54'30.2"W), Rio Preto sub-basin, Rio Itanhaém coastal basin, Itanhaém, southeastern Brazil.

Results and Discussion

Following the coordinates provided for the species' type locality, given in Costa (2008) (24°13′8.9′S, 46°55′24.7′W), we made an unsuccessful effort to sample *L. itanhaensis*. However, to find the exact pool in the dense moist forest environment at these coordinates proved challenging, as a significant portion of the surrounding area had already undergone deforestation and urbanization. Nearby, we identified an area undergoing slight deforestation and marked by open trails, where other temporary pools were discovered (Figure 2), with the species (Figure 3) recorded in one of them in February 2024. We also encountered the species in a roadside ditch in March 2024, which was not in close proximity to its designated type locality (Figure 2). In total, five males were sampled, taken and measured (Table 1), four at the temporary pool and one at the roadside ditch. The physicochemical and structural variables of both environments where the species was found are presented in Table 2. The characteristics of the temporary pool closely resemble the type locality, featuring an acidic pH, shallow depth and situated within the dense rainforest (Costa 2008). However, the roadside ditch exhibited a higher pH value compared to the pool (Table 2), potentially attributed to its predominant source of rainwater rather than flooding from the black water stream, and it also presented a greater depth.

The additional species in both environments also match those listed by Costa (2008) description of other species coexisting with *L. itanhaensis*. Together with *L. itanhaensis*, individuals of *Callichthys callichthys* (Linnaeus, 1758), *Atlantirivulus santensis* (Köhler, 1906), *Mimagoniates lateralis* (Nichols, 1913), and another critically endangered species with few records for the area, *Rachoviscus crassiceps* Myers, 1926 (Santos et al. 2018, Costa et al. 2024), were sampled at both sites.

The specimens sampled in this study, 13.5 to 19 mm standard length (SL), were smaller than those reported by Costa (2008), 16.2 to 21.8 mm SL. The rediscovery of the species in the area near the type locality is very auspicious, but maintains concern about its conservation due to the ongoing deforestation process in the area. This highlights the urgent need for emergency measures to safeguard these temporary habitats and this and other endangered species (e.g. *Rachoviscus crassiceps*; see Costa et al. 2024) associated with these threatened and sensitive environments. Furthermore, additional research is required to ascertain whether species such as *L. itanhaensis* have resorted to roadside ditches as a final recourse in these environments undergoing deforestation and urban expansion.

The rediscovery of the species provides crucial information, indicating that it is not regionally extinct as suggested by Pavanelli et al. (2018), and urging future monitoring and conservation efforts. The primary threats to the species come from urbanization and its appeal in the aquarium market, contributing to a substantial impact on its habitat (Costa 2009, Pavanelli et al. 2018). This finding, coupled with the proximity to the road and ongoing land deforestation around the temporary pool and roadside ditch, underscores the critical need for urgent monitoring and protection of this habitat. Comprehensive inventories are essential to better understand the distribution area of the species (Oyakawa et al. 2009). We hope that the rediscovery of the species will catalyze immediate conservation actions at this new location. Also, we suggest that future work measure the occupancy probability for the species, either through spatial, seasonal and annual monitoring, or through less invasive methods such as environmental DNA, making it possible to better understand whether its long absence is due to the sampling effort employed or to the state of the population.

Figure 2. Temporary pool (A) with deforested surroundings (B) and roadside ditch (C) where *Leptopanchax itanhaensis* individuals were sampled.

Figure 3. Male specimen of *Leptopanchax itanhaensis* (DZSJRP 24846), with its photograph being taken immediately after sampling next to the roadside ditch in Itanhaém, southeastern Brazil. Total length = 24.5 mm. Standard length = 19 mm.

Table 1. Morphometric data for *Leptopanchax itanhaensis* (N = 5) males found at the temporary pool and at the roadside ditch in the Rio Preto sub-basin. SD = Standard deviation.

Morphometric	Minimum	Maximum	Mean	SD
Total length (mm)	16.0	24.5	18.4	3.26
Standard length (mm)	13.5	19.0	15.4	2.21
Weight (g)	0.027	0.143	0.063	0.045
Body depth (mm)	1.94	3.16	2.62	0.48
Head depth (mm)	1.83	2.42	2.17	0.23
Head length (mm)	2.56	4.20	3.25	0.62
Eye diameter (mm)	1.12	1.80	1.32	0.28
Caudal peduncle depth (mm)	0.95	1.90	1.32	0.38
% standard length				
Body depth (%)	14.37	19.75	17.03	2.06
Head length (%)	18.96	23.85	21.08	1.96
% head length				
Eye diameter (%)	33.53	44.92	40.83	4.36

Table 2. Structural and physicochemical variables of both environments where *Leptopanchax itanhaensis* individuals were found. TP = Temporary pool. RD = Roadside ditch. The average values of the variables were taken in February 2024 for the temporary pool and in March 2024 for the roadside ditch.

Variables	TP	RD
Distance to the closest road (m)	22.09	2.40
Maximum length (m)	2.63	7.34
Maximum width (m)	0.70	2.24
Maximum depth (m)	0.11	0.49
Temperature (ºC)	27.13	25.6
Dissolved oxygen (mg/L)	0.90	3.71
pH	4.33	5.8
Turbidity (NTU)	87	75
Conductivity (mS/cm)	0.092	0.073

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Amanda Selinger: data collection; photographs; manuscript preparation and revision.

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Francisco Langeani: specimens' identification and deposit; manuscripts preparation and revision.

Rafael Mendonça Duarte: conceptualization; data collection; manuscript preparation and revision.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Data Availability

All data are available in the paper.

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