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Habitat and Conservation Assessment of Annual Killifishes of the Genus *Xenurolebias* (Rivulidae: Cynolebiinae) from Coastal Floodplains, Including the First Record South of the Rio Doce, Southeastern Brazil †

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Abstract

The Atlantic Forest coastal tablelands is home to a wide diversity of small-sized freshwater fish sharing a biogeographically congruent distributional pattern. The annual killifish *Xenurolebias* are among these remarkable inhabitants in the riparian wetlands with four species in the area. *Xenurolebias pataxo* are located between the Jucuruçu and Mucuri rivers, geographically replaced by *Xenurolebias myersi* at the Riacho Doce and the Rio Itaúnas, and, further south, *Xenurolebias cricarensis* are in the floodplains of the Rio São Mateus and *Xenurolebias izecksohni*, living in temporary environments of the Rio Barra Seca, north of the Rio Doce. Due to their restricted habitat in the lowlands, these annual killifish could all be designated as a target species, providing benefits in the conservation of riparian wetlands. However, less is known about their habits and real distribution. Otherwise, the accelerated habitat loss is worrisome. Two of these species are threatened with extinction on Red Lists; one is Near-Threatened; and one is Data-Deficient. After ten years since the last species description, our investigations revealed a fifth species, the first recorded south of Rio Doce. The present contribution aims to characterize the occupied habitat by each species within the temporary environments and present an updated distributional data on these fish. Additionally, a new *Xenurolebias* is described, the first one recorded south of Rio Doce.

Keywords: Atlantic Forest; Rio Riacho; swamp; cloud fish; endemism

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1. Introduction

From the Rivulidae fish family, the Cynolebiinae subfamily includes annual species, or cloud fish, which complete their life cycle regulated by the rainy season. Their lives are short, quickly reaching sexual maturity and dying during the dry season, when the pools that are their habitat dry up. The eggs remain in a state of diapause in the damp mud at the bottom of the puddle and hatch during the rainy season [1]. However, some aquatic systems can retain deeper pools for longer periods, thereby allowing the persistence of adults in these less hydrologically unstable habitats [2,3].

The Atlantic Forest in Eastern Brazil has certain stretches of mild relief, comprising a phytophysiognomy called coastal tablelands, between southern Bahia and northern Espírito Santo. This area is full of small independent streams, lakes, swamps, and temporary flooded environments, and these habitats are home to a wide diversity of small-sized fish, often endemic [4–6]. By independent streams we mean small river basins flowing directly to the ocean with no connection to a large river basin. This is quite a common condition in the landscape of the coastal tablelands, due to the almost plain relief. In mountainous river basins along the Atlantic Forest, a common condition is a river capture towards neighboring river basins.

The annual killifish *Xenurolebias* are among these remarkable inhabitants in the riparian wetlands with four species restricted to the coastal tablelands. These fish appear only in brief periods of the year [7]. They live in pools of dark, reddish, and acidic waters, in the restinga, or on the edges of forests, in the coastal plains of the north of Espírito Santo to the north of the Doce River [2]. The habitats of these endemic species are sometimes ephemeral wetlands that require special conservation efforts.

The distribution of each taxon is linked to the lowlands of a certain river basin on the coastal tableland bioregion [2]. In extreme southern Bahia, *Xenurolebias pataxo* can be found [8], between the Jucuruçu and Mucuri rivers, geographically replaced by *Xenurolebias myersi* [9] between the Riacho Doce and the Itaúnas river, in the extreme north of Espírito Santo, further south, *Xenurolebias cricarensis* [8], and in the floodplain of the Rio São Mateus and *Xenurolebias izecksohni* [10], which occurs in temporary environments of the Rio Barra Seca, north of the Doce River. Due to their restricted habitat in the lowlands, the annual killifish of the *Xenurolebias* could be designated a target species, providing benefits in the conservation of riparian wetlands in the Atlantic Forest. In recent decades, species of *Xenurolebias* have been the focus of taxonomic review and molecular studies [8,11]. Despite this growing interest, knowledge about these annual fish populations and their environmental conditions and preferences is still poorly known. Data on geographic distribution is often limited to that appearing in their original descriptions [2]. Less is known about them, their habits, real distribution, and human current impacts on their populations.

Two of these species are threatened with extinction on Red Lists: one is Near-Threatened; one is Data-Deficient [12]. Ten years after the last species description, our investigations revealed a fifth species, the first recorded south of the Rio Doce. These fish, inhabiting the swamp areas of the Rio Riacho, are distinct from their geographically closest congener on the Rio Barra Seca swamps, and they are revealed to be a new taxon.

The present study aims to update distributional data on these fish, evaluate their habitat, and describe a new species in the lowlands south of the Rio Doce.

2. Materials and Methods

2.1. Study Area

The coastal tablelands between northern Espírito Santo and southern Bahia, within the larger Atlantic Forest biome, are characterized by their relatively flat-to-wavy terrains, deeply incised valleys, and unconsolidated sedimentary deposits [13]. These tablelands, formed by the Barreiras Formation, are a significant source of sediment for the coastal zone and are used for agriculture, particularly coffee crops and fruits. The phytophysiognomy of the region is composed of ombrophylous dense forest, a formation of the Atlantic Forest. The soils are formed by sediments of the Barreiras Formation and are typically well-drained. Latosoils and sandy soils are covered by dense forest and pioneer vegetation [14,15]. The climate in the region is Am (tropical monsoon), according to the climatic classification system (Köppen–Geiger classification). The annual average temperature is 25 °C, with

averages of 28 °C in summer and 21.4 °C in winter. The average annual rainfall is 1354 mm, with rainy season between October and April.

Along lowlands, these tablelands include coastal ecosystem formations of vegetation such as restinga, muçununga. The restinga corresponds to vegetal formations over sandy deposits along coastal plains. These environments present variable vegetation, according to proximity of sea, and they contain herbaceous-shrubby, arbustive or arboreal formations, including species such as *Clusia* spp., *Vismia guianensis*, and representatives of the *Myrtaceae* family [16,17]. These coastal plain formations also include the muçununga environments, quite similar to the restinga, distinguished by their soil composition with thick layers of sandy sediments, spodosols (typically acidic soils). Vegetation is also variable, ranging from grassy to herbaceous-shrubby or arboreal, including species such as *Byrsonima sericea*, *Tapirira guianensis*, and *Marcetia taxifolia* [18]. Water accumulates in flooded areas that, due to the concentration of large quantities of leaves and tannin, display a tea-like or amber coloration. In these environments the freshwater drainages are marked by small streams, flowing in slow currents, puddles, and temporary pools. These pools are dynamic environments, with water levels and durations varying according to rainfall and additional climate factors, resulting in highly heterogeneous and fragile ephemeral wetlands [19]. The temporarily flooded environments support unique plant and animal communities, mostly endemic, adapted to these fluctuating conditions [7,14].

Coastal floodplains along river basins of Jucuruçu, Itanhém, Peruípe, Mucuri, Riacho Doce, Rio Itaúnas, Rio São Mateus, Rio Doce, and Rio Riacho are temporally subject to floods. The hydrological dynamics of these lowlands are markedly complex [20]. The lotic systems are peculiar with large river systems and also small rivers, flowing in slow current. In lowlands there are plenty of lentic environments such as lake systems, ponds, puddles, and swamps. These areas experience variations in water levels due to precipitation and topography, leading to diverse aquatic habitats.

2.2. Sample Design and Data Collection

In search of fish populations, 22 expeditions and three field campaigns were conducted between December 2021 and March 2025, including visits to the type localities of the species *Xenurolebias*, as well as the exploration of additional environments. Each sampling point was located by GPS (Global Positioning System) and plotted on a map using GPS Trackmaker Professional 4.9 [12]. Distance and cartographic area calculations were performed based on maps created in the same program. During the field activities, the Google Maps and Timestamp Camera Free applications were also used to support area identification and location data recording. QGIS 3.34.4 software was used to create the maps. The collection points were photographed and characterized according to environmental conditions and abiotic parameters. To systematize these data, a field spreadsheet was used to record information about the environment, water, and fish observed. Abiotic data were collected using an AK88 multiparameter meter (AKSO® Electronic Products, São Leopoldo-RS, Brazil), which measures pH, temperature, and humidity.

For sampling, exclusively artisanal sieves and scoops, with standardized measurements of 90 cm × 60 cm, were used as fishing equipment. This equipment proved to be more suitable for shallow environments, allowing them to reach the bottom of the pools, resulting in more effective and accurate sampling. Small pools (maximum 3 m^2) and shallow pools (maximum 60 cm deep) were fully explored, with intense incursions. However, in larger pools, a sampling standard was established every one meter, for a total period of 10 min per sampled point.

The collected material was fixed in 10% formalin and deposited in the ichthyofauna for cataloging. Voucher specimens of the new taxonomic species were collected and deposited

in the ichthyological collections of the National Museum (MNRJ) and the Mello Leitão Biology Museum (MBML). All captured individuals were euthanized before fixation, and field methods were approved by the Animal Use Ethics Committee of the Federal University of Espírito Santo (CEUA-UFES; application number: 008/23).

Morphometric and meristic data were taken according to [21]; measurements are presented as percent of standard length (SL), except for those related to head morphology, which are expressed as percent of head length (HL). Fin-ray counts include all elements.

The Extent of Occurrence (EOO) and Area of Occupancy (AOO) [22] were estimated for each *Xenurolebias* species using the online tool GeoCAT (Geospatial Conservation Assessment Tool). GeoCAT is an open-source platform developed to support Red List assessments according to International Union for Conservation of Nature (IUCN) criteria.

The georeferenced records used were obtained from the collection databases and the sampling points conducted in this study, supplemented by historical records verified in the literature. Each location was entered using GPS coordinates in decimal degrees.

The AOO was estimated based on a standard 2 × 2 km grid, as recommended by the IUCN (2022) [22]. The EOO was calculated using the minimum convex polygon. Both estimates were used for a preliminary assessment of the conservation status of each species based on criterion B of the IUCN Red List [23]. The EOO is one of the key metrics adopted to determine a species' conservation status, and the same methodology was adopted and standardized by the Brazilian Ministério do Meio Ambiente in the evaluation of priority areas for conservation of annual fish of the Rivulidae family [24]. The threat vectors are according to the IUCN Red List and correspond to any factor that causes the decline of species populations and may contribute to risk of extinction, being classified into several categories to guide conservation actions. The selection of threat vectors follows the Red List Guidelines in [23].

3. Results and Discussion

3.1. Xenurolebias tupinikin sp. nov. (Figures 1–7, Tables 1 and 2)

Holotype. Location characteristics are as follows: MNRJ 56004, 23.4 mm SL, Brazil, Espírito Santo State. Aracruz, Rio Riacho basin. Swamp area on Sertão do Riacho stream, −19.73992 S −40.04357 W, 26 January 2025, B. Pinheiro.

Paratypes. Location characteristics are as follows: MNRJ 56005, 9, 16.4–23.4 mm SL, Brazil, Espírito Santo State. Aracruz, Rio Riacho basin, swamp area on Sertão do Riacho stream, −19.73992 S −40.04357 W, 26 January 2025, B. Pinheiro, collected with the holotype.

Diagnosis. *Xenurolebias tupinikin* is distinguished from remaining congeners by adult males presenting in life a dark blotch overlapping the median portion of the last stripe on the caudal peduncle (Figure 2). It further differs from congeners, except *X. myersi*, by presenting the dorsal and anal fin borders with a black outline. Additionally, it is distinct from *X. myersi* by a body depth of 25.9–27.9 in SL (vs. 29.0–31.4 in SL).

It differs from *X. izecksohni* and *X. cricarensis* by the caudal fin in males with 5–6 bars (vs. 7–14 bars) and by male head depth 64.5–76.9% of HL (vs. 81.2–85.6% of head length in males). It further differs from *X. pataxo* and *X. myersi* due to no yellow spots on the distal half of the dorsal fin in males (vs. yellow spots present).

Description. Morphometric data are available in Table 1. Maximum adult size is 23.9 mm SL. The body is slender, sub-cylindrical anteriorly, slightly deeper than wide, and it is compressed posteriorly; the greatest body depth is at the level of the pelvic-fin base. Dorsal and ventral profiles are gently convex from the snout to the end of the dorsal and anal-fin bases, nearly straight on the caudal peduncle. Jaws are short, and the snout is blunt. The eye is small, positioned on the dorsal portion of the head side. The extremity of the dorsal fin is pointed and long in males, rounded to slightly pointed in females.

The tip of the anal fin is pointed in males, rounded in females; in males, there are one or two filamentous rays on the tip of the dorsal and anal fins reaching vertically between the base and middle of the caudal fin. The caudal fin is rounded or sub-lanceolate in shape. The pectoral fin is elliptical, and the posterior margin reaches vertically between the base of the third and sixth anal-fin rays in males, reaching the urogenital papilla in females. The pelvic fin is small, with the tip reaching the base of the second or third anal-fin ray in males and the base of the third anal-fin ray in females. Pelvic-fin bases are medially united. The dorsal-fin origin is vertical through the base of the eighth or ninth anal-fin ray in both sexes. There are 17 dorsal-fin rays in males, 12–13 in females; there are 21 anal-fin rays in males, 21–23 in females; 21 caudal-fin rays; 13 pectoral-fin rays; and 6 pelvic-fin rays. Scales are small, cycloid. The body and head are entirely scaled, except the ventral surface of the head. Body squamation extends over the anterior 25% of the caudal-fin base; few scales slightly extend across the middle of the anal-fin base; no scales are on the dorsal-fin base. Scales are arranged in a regular transverse pattern. There are two small supraorbital scales. There are 27 longitudinal series of scales and 9 transverse series of scales. There are scale rows around caudal peduncle 12. The contact organ is located on each scale of the ventral portion of the flank in males. Minute papillate contact organs are on two dorsal-most rays of the pectoral fin in males. There is a single neuromast on each scale of the lateral line; 2 neuromasts on the caudal-fin base. Cephalic neuromasts: supraorbital 10–12, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 2 + 16–18, preorbital 3, otic 1, post-otic 2, supratemporal 1, median opercular 1, ventral opercular 2, pre-opercular plus mandibular 23–24, lateral mandibular 5–7, and paramandibular 1.

Live coloration. Male (Figure 3A). The side of the body is brownish-yellow, with 12–13 dark brown bars; the caudal peduncle has a dark spot united to the posterior-most bar. The dorsum is pale brown. The center is pale pink. The opercular and infraorbital region is yellowish, with two diffuse dark bars. The iris is light yellow with a dark reddish brown bar in the middle part. The dorsal fin is dark brown with scattered irregular marks, and the fin margin becomes dark towards its tip. The anal fin ranges from dark brown to dark red, with irregular marks. The caudal fin is pale yellow, with 5–6 dark irregular bars. The pectoral fin is hyaline with a brownish border. The pelvic fin is light brown.

Female (Figure 3B). The side of the body is light brown, with 10–11 dark gray bars; one or two black blotches are on the anterocentral part of the flank. The dorsum is pale brown. The center is light pink. The opercular region is yellowish. The iris is light yellow, with a dark brownish bar. Unpaired fins are hyaline with small dark brownish spots. Paired fins are hyaline.

Distribution. The Rio Riacho corresponds to the first independent coastal river basin south of the Rio Doce. Flowing along lowlands, the Rio Riacho crosses savannah formations as restinga and muçununga. Its aquatic environments have predominantly sandy substrate and banks covered by riparian vegetation in different states of conservation. The largest tributary of Rio Riacho corresponds to the Rio dos Comboios, which runs in parallel to the coastline until meeting the Rio Riacho close to its mouth.

The new species is known from three localities, in temporary pools near two tributaries of the Rio Riacho (Figure 4-P01 to P03). It was found in the Sertão do Riacho stream and as well as in the neighboring stream, the Caipora stream, which are all contributors to the right margin of the Rio Riacho, a coastal river drainage south of the Rio Doce, north-central Espirito Santo (Figure 4).

Ecological notes. The Sertão do Riacho isolated pool, identified as an extensive swamp area, with approximately 2 km^2. It is completely covered by dense emerging vegetation, formed mainly by grasses and ferns. The substrate is formed by emerging vegetation, decomposing plant material, and sand (Figure 5). Abiotic information is as follows: dark

tea-colored waters in a lentic environment; water depth in the pool is between 40 cm and 60 cm; humidity: 60%; water temperature: 27.1 °C; and pH: 4.3–6.2 (Table 2).

The swampy area at the type locality (Figure 4-P02) is about 100 m from point 1 (Figure 4). It features dense emergent vegetation, marginal vegetation, and floating macrophytes. The swamp showed an abundance of *Xenurolebias tupinikin*, adults and juveniles, scattered throughout the swampy area, found mainly under vegetation.

Sampling was carried out in various parts of the pool, between edges and the central part, with some limitations due to the dense vegetation that emerged from the bottom of the pool. In P02 (Figure 4), only adult individuals, three females and one male, were captured for recording. Additional specimens were immediately returned to the environment. As for the accompanying fauna, aquatic insects in a wide variety and a specimen of *Callichthys callichthys* were captured.

Etymology. The specific name is a reference to the Tupiniquim Indigenous people, inhabitants of lowlands in the central north of Espírito Santo. On the left margin of the Rio Comboios is the Indigenous land Comboios (Figure 4, green stripes near coast), which corresponds to the nearest human occupation relative to the environments inhabited by these fish.

Conservation concerns. The estimated extent of occupancy (EOO) of the population found in the Rio Riacho basin was defined as 0.097 km^2, and the area of occupation is 0.136 km^2 (Figure 6). From May until September, the pools where the rivulids were captured become dry. The distance from the pool to the sea is approximately 4 km in a straight line (Figure 4). The Sertão do Riacho pool is near the road to the village of Riacho and quite anthropized. At the site, garbage dumping points (solid waste) and debris (civil construction waste) were identified. Threat vectors to *Xenurolebias tupinikin* populations, according to [23] criteria, include habitat destruction due to agropecuary and *Eucalyptus* crops (vector 2.2.2), sand extraction (vector 3.2.1), human disturbance (vector 6.3), pollution and domestic garbage (vector 9.4.1), climate change (vector 11.1), and extreme drought (vector 11.2).

Remarks. The Rivulidae family is considered one of the most endangered among freshwater fishes [25], an even more critical condition for species with an annual life cycle, which depend directly on the rainfall regime to complete their development. These fish inhabit temporary environments that are highly susceptible to fragmentation and degradation, which makes their populations extremely vulnerable. Such factors should be carefully considered in the conservation status assessments of the species. The *Xenurolebias tupinikin* is recognized as a member of genus [8] by possessing sexual dimorphism in a number of dorsal and anal-fin rays and numerous supraorbital neuromasts (nine or more) and the three synapomorphies uniquely shared by species of *Xenurolebias*: presence of filamentous rays on the posterior margin of the anal fin in females, caudal fin sublanceolate to lanceolate and asymmetric due to an expansion of the ventral portion of the fin in males, and a dark reddish bar on the posterior portion of the caudal peduncle posteriorly extending over the dorsal and ventral margins of the caudal fin in males [26].

Figure 1. *Xenurolebias tupinikin*: MNRJ 56004, male, holotype, 23.4 mm SL; MNRJ 56005, female, paratype, 35.4 mm SL. Pool at flooded area on Sertão do Riacho stream, Aracruz, Espírito Santo, Brazil.

Figure 2. Diagnostic features for *Xenurolebias tupinikin*. Dark blotch overlapping the median portion of the last stripe on the caudal peduncle (arrows).

Figure 3. *Xenurolebias tupinikin*: (**A**) Male. (**B**) Female.

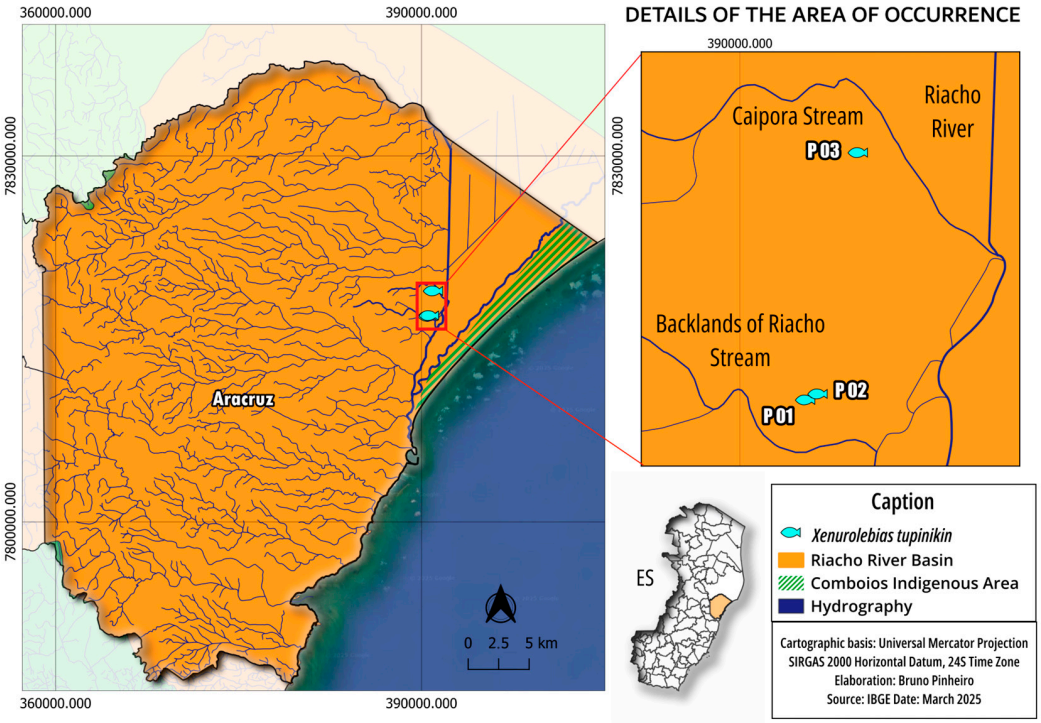

Figure 4. Sampling sites for *Xenurolebias tupinikin*. P01—Sertão do Creek Stream. P02—Type locality. P03—Temporary pool in the wetlands of Caipora Stream. The area with green stripes between the banks of the Rio dos Comboios and the ocean corresponds to the Comboios Indigenous Land of the Tupiniquim people.

Figure 5. Sertão do Riacho stream. Type locality of *Xenurolebias tupinikin*.

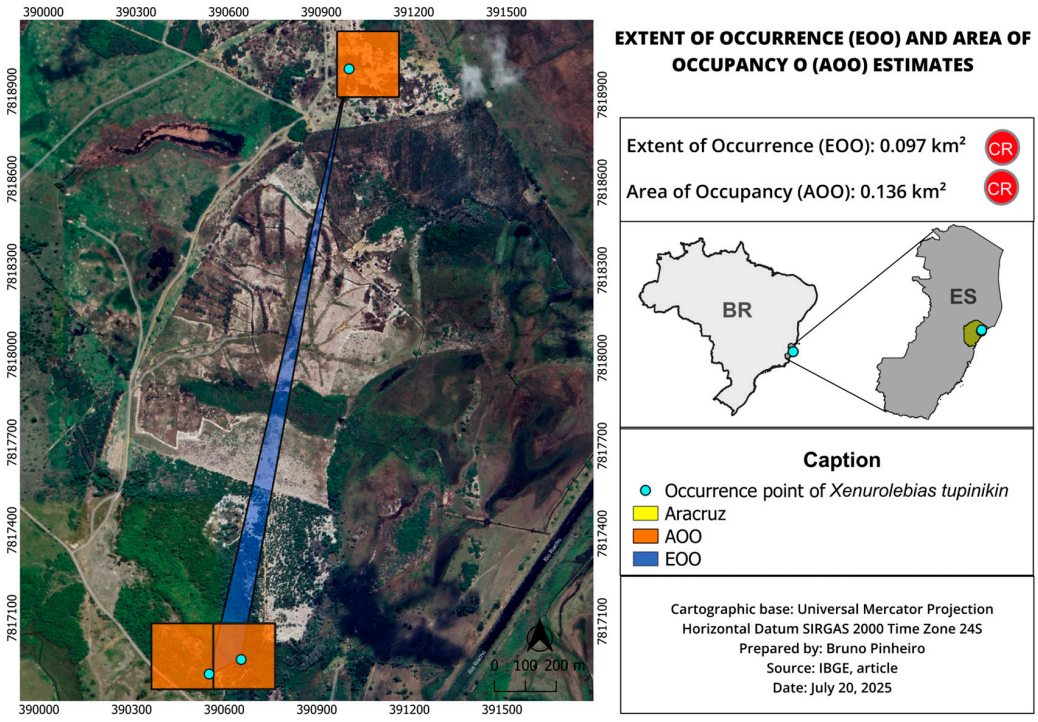

Figure 6. Extent of occurrence (EOO) and area of occupancy (AOO) estimates for *Xenurolebias tupinikin*. Localities correspond to those marked in map in Figure 4.

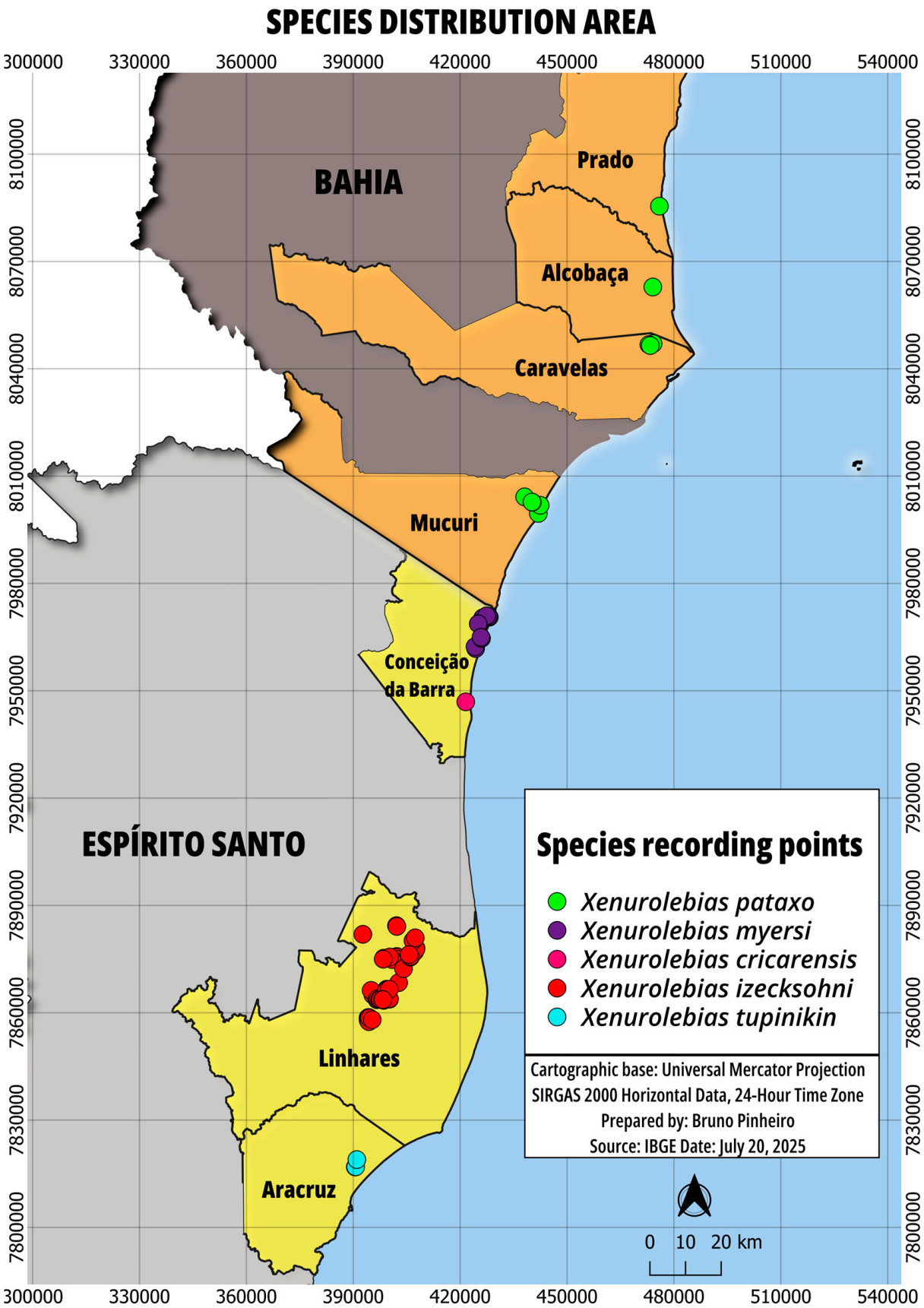

Figure 7. Location of the distribution area of *Xenurolebias* species, on the coastal plateaus between southern Bahia and northern Espírito Santo. Municipalities in Espírito Santo are marked in yellow, and municipalities in Bahia are marked in orange. Scale bar: 20 km.

Table 1. Morphometric data of *Xenurolebias tupinikin*. Holotype included in the ranges of males. **n** = number of specimens examined.

	Xenurolebias tupinikin		
	Holotype Male	**Male (n = 5)**	**Female (n = 10)**
Standard length (mm)	23.3	20.3–23.3	16.5–23.9
Percentages of standard length			
Body depth	26.9	25.9–27.9	19.0–32.9
Caudal peduncle depth	14.3	11.4–14.4	6.6–14.7
Predorsal length	59.0	56.6–64.1	48.8–73.9
Prepelvic length	44.0	42.7–49.4	44.1–56.2
Length of dorsal-fin base	24.2	24.2–29.2	09.1–16.8
Length of anal-fin base	51.0	47.6–56.1	17.8–26.1
Caudal-fin length	40.7	37.3–40.7	29.2–39.5
Pectoral-fin length	32.9	27.8–33.2	15.8–29.7
Head length	29.9	29.2–31.5	28.5–33.8
Percentages of head length			
Head depth	76.8	64.4–76.8	61.27–85.5
Lower jaw length	25.5	18.4–25.5	15.1–21.6
Eye diameter	32.2	31.5–46.1	10.8–34.8

Table 2. Abiotic data for *Xenurolebias* species.

Environmental Features	**Values Per Species**				
	X. myersi	***X. izecksohni***	***X. pataxo***	***X. cricarensis***	***X. tupinikin***
Depth (cm)	40–110	20–70	30–50	50–60	30–60
Temperature (°C)	24–28	22–37	25	24.6	26.5–29.2
pH	3.5–5.6	3.5–5.6	4.2–5.2	5.6	4.3–6.2
Humidity (%)	60–80	60–88	43–60	68	54–60

3.2. Xenurolebias izeckshohni (Figures 8–11)

Xenurolebias izecksohni was described for the Barra Seca river basin, at the locality of Canto Grande, Farias, within the Vale Natural Reserve (RNV), a private preserved area [8,10]. Until now, the distribution of the species has been limited to the Barra Seca river basin. Between 2023 and 2025, field campaigns were carried out in search of populations. New populations were found at the Doce River basin, considerably expanding its area of occurrence to include two river basins, Doce and Barra Seca (see the map in Figure 10). The collections were carried out in different periods, covering both the dry and rainy seasons, which allowed the measurement of important data hitherto unknown for the species. It was observed that the life cycle of the species is configured as biannual, not annual, as expected. That is, throughout the year, the puddles dry up and reestablish themselves twice, usually between November and March, the first cycle, and between May and August, the second, and there may be variations according to the climatic conditions of each year. In addition, it was found that puddles completely exposed to the sun dry out more quickly, while those with vegetation cover tend to persist for longer and may even remain flooded throughout the year, which maintains populations alive in these environments.

Most of the new populations of *Xenurolebias izecksohni* registered are distributed in private areas, including rural properties and lands of the Linhares Agroindustrial Plant—LASA, an ethanol-producing company. In addition to places around the RNV, in the vicinity of the type locality. The number of individuals per pond varied between a few specimens (one to five) and concentrations of more than fifty individuals. The morphometric analysis revealed a considerable variation in the standard length (SL), with values higher than those recorded in the literature for the species (30–43 mm) [8]. Males with values up to 54.24 mm

and females with values up to 41.36 mm were measured, evidencing the occurrence of specimens larger than those previously described. Behaviors observed in the field indicate a preferential occupation of the edges of the puddles by *Xenurolebias izecksohni*, especially under vegetation cover or between submerged leaves. The color of the individuals varied according to the shade of the water, which, in many cases, presented a dark color due to the presence of tannins from the decomposition of organic matter. Under these conditions, the fish showed equally darkened coloration, which may indicate phenotypic plasticity and adaptation to the environment, possibly related to camouflage mechanisms and protection against predators.

Native Vegetation and Landscape. In view of the new records, it was possible to verify that the habitats occupied by *Xenurolebias izecksohni* present considerable variation in their structural aspects. Individuals have been found in different types of temporary aquatic environments, including puddles, ponds, and even intermittent streams. Such environments varied in terms of vegetation cover, with conditions of total exposure, partial shading, or complete vegetation cover. In addition, the composition of the vegetation was also diverse, with records of marginal, floating, and emerging plants. The puddles, specifically, showed great variability in terms of morphology (circular, elongated, and amoeboid shapes), substrate (sandy, leafy, or mixed), depth, and color of the water, revealing a considerable ecological plasticity of the species. Puddles in open areas exposed to the sun showed clear waters and emergent herbaceous or aquatic vegetation, while those in areas shaded by shrub or forest vegetation exhibited darker waters and substrate rich in organic matter. Abiotic information: Dark tea-colored waters in a lentic environment. Water depth at the pool measured between 20 cm and 70 cm; humidity: 60–88%; water temperature: 22–37 °C; and pH: 3.5–5.6 (Table 2).

Similar to what occurs with many species of rivulids (15–16), *Xenurolebias izecksohni* occupies ephemeral habitats, with very peculiar characteristics, becoming inhospitable to most fish species. This condition was observed throughout the expeditions, in which records of coexistence of *X. izecksohni* with other species of ichthyofauna were rare. In less than 10% of the sampled points, specimens of other species, such as *Astyanax* spp., were collected. *Hoplias malabaricus*, *Hoplerythrinus unitaeniatus*, and *Callichthys callichthys* were usually in larger, newly formed puddles and close to springs or perennial watercourses. In these cases of coexistence with other fish, the density of *X. izecksohni* was considerably reduced, suggesting a possible negative impact of the presence of predators or competition for resources. In contrast, in the smaller, more isolated pools, in which *X. izecksohni* was often the only species recorded, the population density was significantly higher, indicating a possible adaptive specialization to environments with low biotic pressure.

In newly formed puddles after rain events, newly hatched fish were recorded, which indicates recent reproductive episodes and reinforces the importance of these temporary environments to serve as a nursery for fry and young individuals replicating the reproductive success of these fish. In addition, an unusual observation was the simultaneous capture of individuals at different stages of development, including fry individuals and adults living in the same environment. Such coexistence is uncommon among rivulids, whose life cycle usually involves synchronization between hatching and periods of temporary flooding, with no overlap of generations [27,28]. These data may indicate local phenological variations or plastic responses to changes in environmental conditions.

Conservation concerns. The estimated extent of occupancy (EOO) of the population found in both Doce and Barra Seca river basins was defined as 247.021 km^2 (more than 5 locations) (Figure 11). The pools where the rivulids occur were full of water at least twice a year. The area of occupation (AOO) is 157.496 km^2, demonstrating a significant expansion of the previously known distribution. *Xenurolebias izecksohni* is listed as “Vulnerable” by

the study of [22]. Otherwise, the new information obtained indicates that its situation may still be delicate for its environments than previously estimated, especially in the face of intense anthropogenic pressure on unprotected private areas, where the species was widely recorded during this study. Threat vectors to *Xenurolebias izecksohni* populations, according to [23] criteria, include habitat destruction due to extensive agropecuary, mainly of cattle (vector 2.3.1), sugarcane and *Eucalyptus* crops (vector 2.2.2), oil extraction (vector 3.1), sand extraction (vector 3.2.1), human disturbance (vector 6.3), climate change (vector 11.1), and extreme drought (vector 11.2).

Figure 8. *Xenurolebias izecksohni*: (**A**) MBML 14205, male, 54.2 mm SL; (**B**) MBML 14208, female, 29.6 mm SL. Isolated pool in flooded area in Barra Seca, Linhares, Espírito Santo, Brazil. Images: Bruno Pinheiro.

Figure 9. Representation of the local riparian. Image: Bruno Pinheiro.

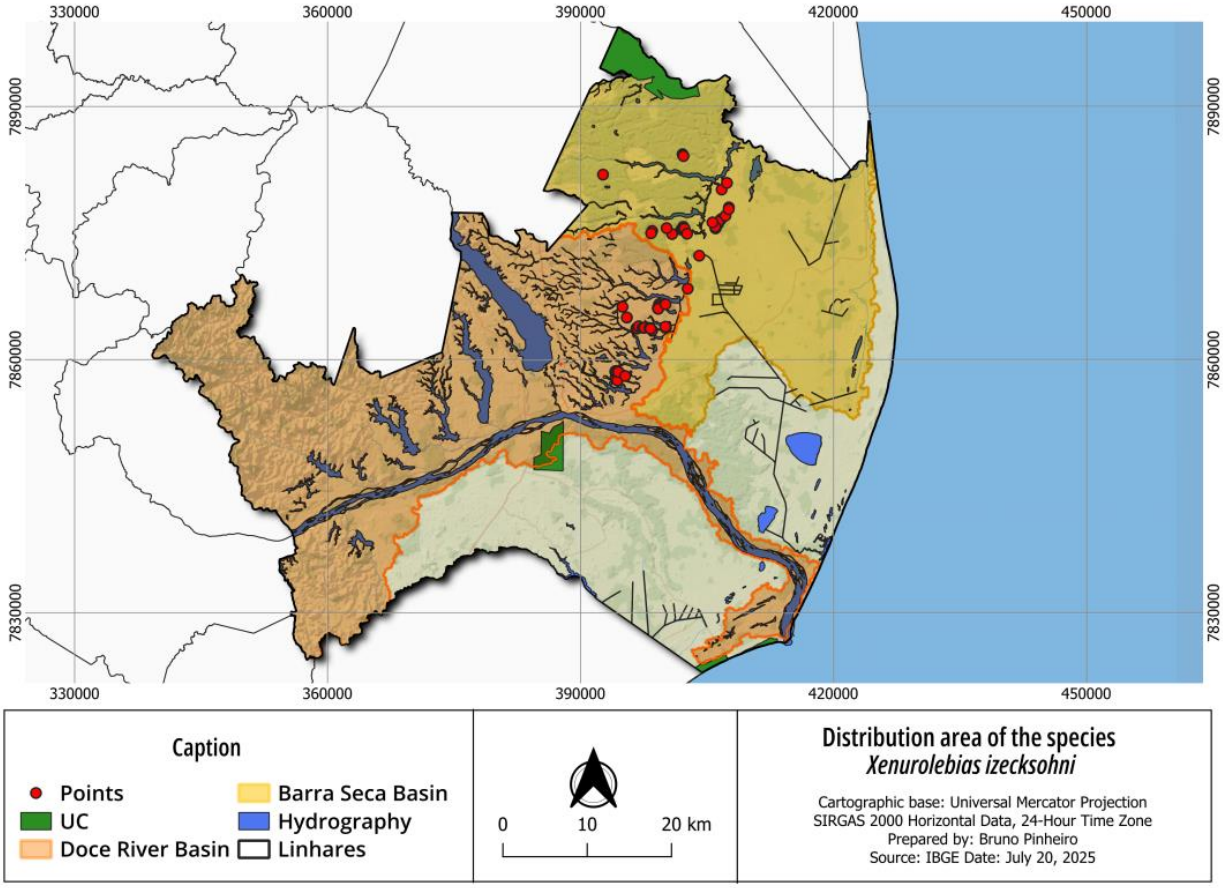

Figure 10. Distribution area of the species *Xenurolebias izecksohni*. Dark blue on left margin of the Doce river corresponds to internal lake systems. Green area corresponds to conservation unit Floresta Nacional de Goytacazes, on right margin of the Doce river. Gray area corresponds to alluvial deposits.

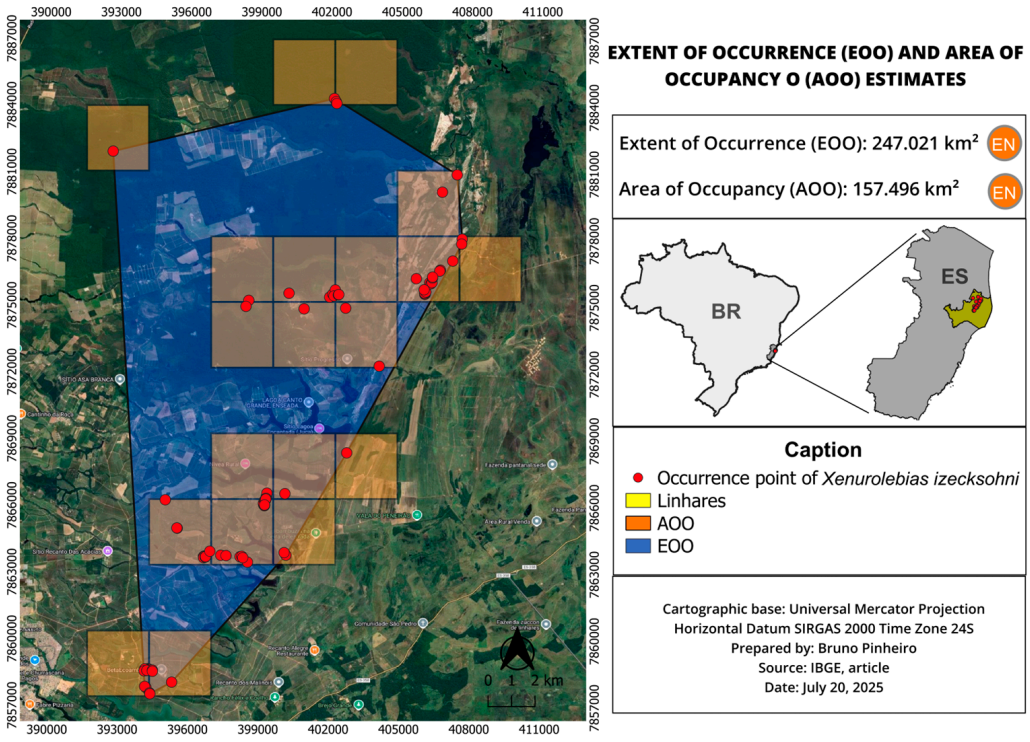

Figure 11. Extent of occurrence (EOO) and area of occupancy (AOO) estimates of *Xenurolebias izecksohni*.

3.3. *Xenurolebias cricarensis* [8] (Figures 12–14)

Xenurolebias cricarensis is known to occur only in its type locality, a temporary marsh located within a farm, close to the Adolfo Serra highway (ES-010) to the city of Conceição da Barra, northern Espírito Santo.

In 2024, two expeditions were carried out in search of the species. During the dry season, in June, an expedition visited the type locality, but no specimens were found, since the environment was completely dry. Other points nearby were visited but were unsuccessful. A second expedition was carried out during the wet season, between 23 and 29 November 2024, in the same region. This time the pond was flooded, and populations were recorded, the first findings since the species description.

During the wet season field campaign, 14 specimens of *X. cricarensis* were collected, along with the accompanying fauna composed of aquatic insects, tadpoles, and mollusks. Specimens of *Oreochromis niloticus* were also captured. Additional areas, with potential occurrence, were visited, but the only one with populations is that single puddle in the sub-basin of the São Domingos stream.

Considering that *X. cricarensis* is dependent on temporary pools, its persistence is directly related to climatic conditions and the integrity of these environments. Degradation can lead to the local extinction of the species, which highlights the need for urgent actions, such as the creation of conservation units and the awareness of landowners about the ecological importance of these systems.

Native Vegetation in the Landscape. The environment in São Domingos is a large temporary swamp, measuring approximately 3 km^2 with dark-colored acidic waters, clay soil, and a substrate with decomposing leaves and branches. These characteristics refer to the initial portion of the puddle, which is easier to access, located next to the road. The bottom portion was not accessible and remains to be investigated. Abiotic information: Dark tea-colored waters in a lentic environment; water depth at the pool ranged between 50 cm and 60 cm; humidity: 68%; water temperature: 24.6 °C; and pH: 5.6 (Table 2).

Conservation concerns. In the Red List criteria, "location" refers to a threat-based area and is different from the notion of locality. A location corresponds to an area where a single development project can rapidly eliminate or severely reduce the population [29]. The estimated extent of occupancy (EOO) of the population found in the São Mateus river basin corresponds to a single location (Figure 14). Only one pool was inhabited by populations. From May until the beginning of the rainy season, the pool was completely dry. Categorized as "Data-Deficient" (DD) by the study of [12], the recent data obtained reinforces that *Xenurolebias cricarensis* needs to be categorized as threatened with extinction. The species has only been found in a single puddle in the sub-basin of the São Domingos Stream, approximately 85 km^2, totaling an approximate occupation area of 0.15 km^2.

This small area is largely anthropized, with the detection of the practice of cattle grazing in the wetlands. Additionally, such a puddle is in the vicinity of the city and subject to urban pressure. This evidence reinforces the urgent need to create protected areas around the local wetland habitats. Coexistence with exotic species, such as tilapia, may also compromise population viability due to competition and habitat degradation. Threat vectors to *Xenurolebias cricarensis* populations, according to [23] criteria, include habitat destruction due to urban expansion (vector 1.1), extensive cattle agropecuary (vector 2.3.1), *Eucalyptus* crops (vector 2.2.2), human disturbance (vector 6.3), climate change (vector 11.1), and extreme drought (vector 11.2).

Figure 12. Couple of *Xenurolebias cricarensis* in field aquarium. Female in front, male behind. Image: Bruno Pinheiro.

Figure 13. Flooded area in the sub-basin of the São Domingos stream at km 16 of the ES-421 Adolfo Barbosa Serra Highway on the left in the direction of Conceição da Barra (type locality *of Xenurolebias cricarensis*). Image: Ronaldo Pinheiro.

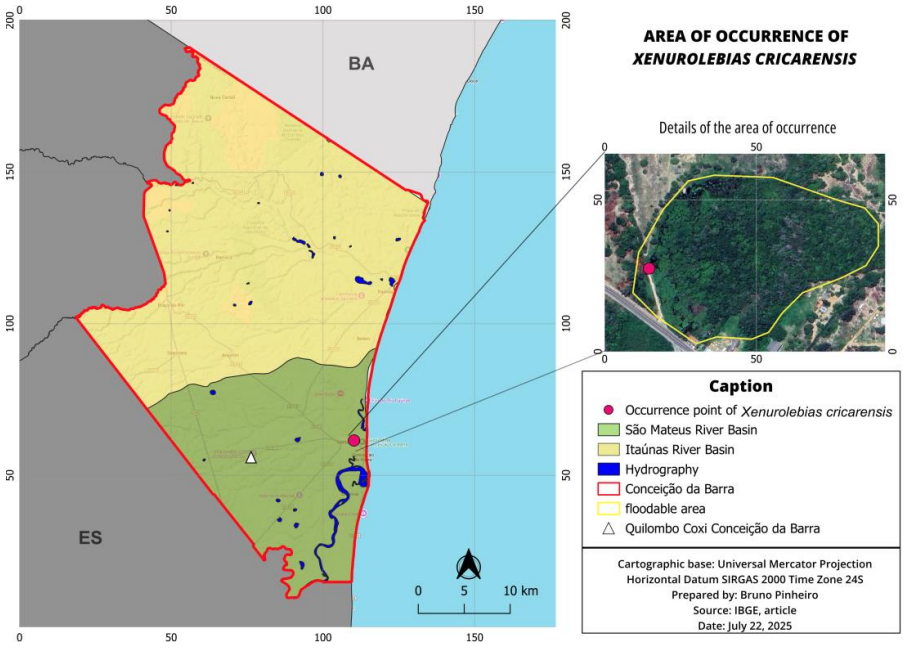

Figure 14. Estimates of EOO and area of occurrence of *Xenurolebias cricarensis*. Pink dot correspond to locality of occurrence. The white triangle upstream of the place of occurrence corresponds to the Quilombo Coxi Conceição da Barra. Dark blue corresponds to main channel of São Mateus river. Light blue corresponds to Atlantic Ocean.

3.4. Xenurolebias myersi (Figures 15–17)

The Itaúnas cloud fish inhabits the sub-basins of the Velha Antônia stream, Moças stream, in the Itaúnas river basin, and additionally in the Limo stream, a tributary of the Doce creek basin [30,31]. These bright-colored fish are sexually dimorphic. They live in peculiar environments, where water is transparent, translucent, in a yellowish or dark orange tone, without turbidity.

Native Vegetation in the Landscape. The species inhabits seasonal swamps in open areas of taboal (*Typha* spp.) or in restinga forests. They are found in temporary freshwater floods, of varying size, from small defined puddles with tens of meters to very extensive swamp areas, with a few square kilometers. Such environments are in the floodplain of the rivers, some very close to the sea, just over a hundred meters from the beach. The puddles are shallow, between 20 and 70 cm deep. The bed of the marsh is composed of a triple layer of substrate: leaf litter, red mud, which occupies about 20 cm or more deep, and then sand underneath. Abiotic information: Dark tea-colored waters in a lentic environment; water depth at a pool ranges between 40 cm and 110 cm; humidity: 60%; water temperature: 27.1 °C; and pH: 3.5–5.6 (Table 2).

Conservation concerns. The estimated extent of occupation (EOO) of the population found in the Itaúnas and Riacho Doce river basins was defined as 14.646 km^2 (more than five locations), and the area of occupation was 6.653 km^2 (Figure 17). The pools where the rivulids occur were full of water at least twice a year. *Xenurolebias myersi* is endemic to coastal lowland swamps and restinga environments in Itaúnas village and the Riacho Doce locality. Categorized as "Endangered", the species is also included in the Action Plan to conserve Rivulidae fish [24].

It is noticeable that few populations were recorded inside the Itaúnas State Park, and most of them are found in environments outside the limits of the park, susceptible to impact, running imminent risks of extirpation. Attention should be given to the village of Itaúnas, which recently underwent several modifications, driven by tourism and immobility pressure, urban expansion, as well as the pressures of monocultural *Eucalyptus*

plantations in its surroundings. Threat vectors to *Xenurolebias myersi* populations, according to [23] criteria, include habitat destruction due to urban expansion and tourist activities (vector 1.3), *Eucalyptus* crops (vector 2.2.2), human disturbance (vector 6.3), and climate change (vector 11.1).

Figure 15. (**A**). *Xenurolebias myersi*, adult male with lanceolate dorsal and anal fins and contrasting vertical bars on flanks. Image: Bruno Pinheiro. (**B**). *Xenurolebias myersi*, adult female with translucent fins and dark dots on flanks. Image: Frederico Pereira. Images in field aquarium. Specimens not preserved.

Figure 16. Velha Antônia stream, near village of Itaúnas. Type locality of *Xenurolebias myersi*. Image: Luisa Sarmento.

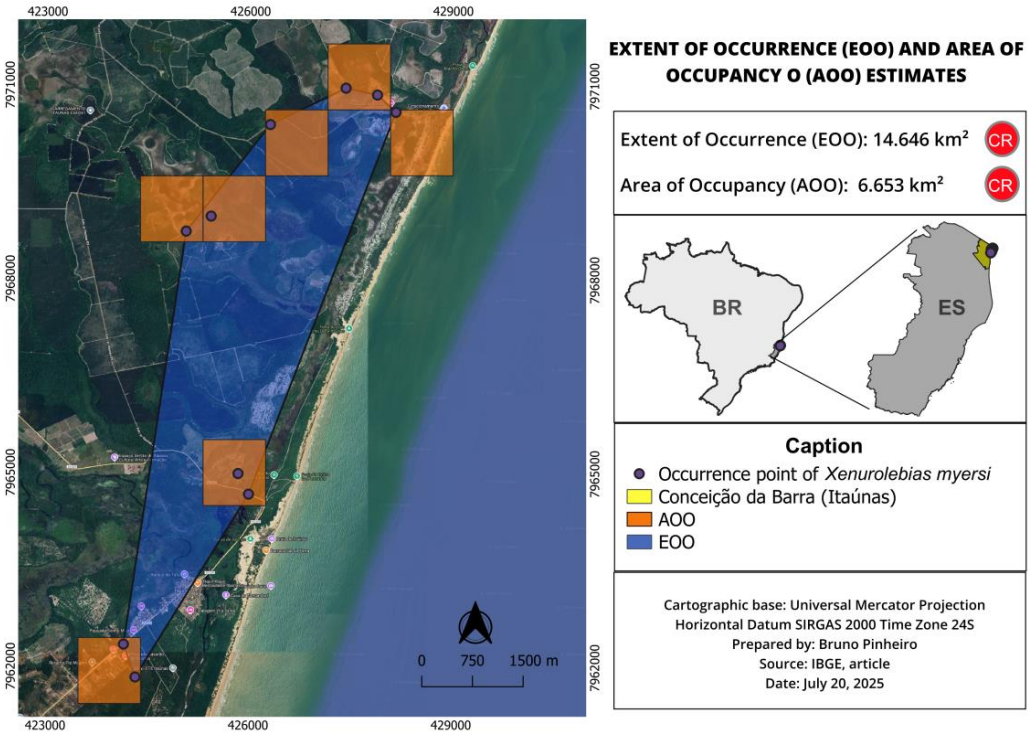

Figure 17. Extent of occurrence (EOO) and area of occupancy (AOO) estimates of *Xenurolebias myersi*. Blue dots corresponds to localities with occurrence.

3.5. Xenurolebias pataxo (Figures 18–20, Table 2)

Xenurolebias pataxo is recorded from a broad coastal plain area along coastal tableland formation at southern Bahia between the Jucuruçu river basin and the Mucuri river basin, about 95 km in a straight line [8]. The area is situated within a tourist zone, which extends to the town of Porto Seguro, one of the most popular tourist destinations in South America. As a consequence, the natural habitats have undergone a great decline since the 1980s, with remarkable habitat loss in the last two decades following intensive urbanization. Populations recorded between 1989 and 1998, situated in the Jucuruçu, Itanhém and Peruípe river basins, as well as some populations previously found around the village of Mucuri close to the Mucuri river mouth, have disappeared. Collecting efforts between Prado and Peruípe were not successful in finding these fish. A population was detected on the Itanhém river lowlands in 2007 (Figure 19).

Conservation concerns. The estimated extent of occupation (EOO) of the population found between the Mucuri and Jucuruçu river basins was defined as 807.259 km^2 (five locations) and the area of occupation as 346.071 km^2 (Figure 20). All records were sampled more than ten years ago. Nowadays the species is found at a single location only, the type locality. Although the original area of occupancy was about 200 km^2, today, *X. pataxo* is found in a small area of about 10 km^2. The abrupt and extensive environmental decline indicates that *X. pataxo* is at least not Vulnerable. Threat vectors to *Xenurolebias pataxo* populations, according to [23] criteria, include habitat destruction due to urban expansion and tourist activities (vector 1.3), extensive cattle agropecuary (vector 2.3.1), *Eucalyptus* crops (vector 2.2.2), sand extraction (vector 3.2.1), pollution and domestic garbage (vector 9.4.1), human disturbance (vector 6.3), climate change (vector 11.1), and drainage of wetlands (vector 12). The whole area is in need of additional investigation, as some are old records from collections (from the Prado municipality).

Based on the estimated extent of occurrence (EOO), area of occupancy (AOO), the degree of habitat degradation, and also field observations, we propose a revision on threat

categories for each *Xenurolebias* species, following the IUCN Red List criteria [23]. A summary of the proposed reclassifications is presented in Table 3.

Table 3. Proposed IUCN Red List categories for *Xenurolebias* species based on field data, estimated EOO, and AOO.

Species	EOO (km²)	AOO (km²)	Current Status	Suggested IUCN Status	Justification (IUCN Criteria)
Xenurolebias tupinikin	0.097	0.136	Not assessed	CR B1ab(iii)	Very restricted range, only three known localities, degraded habitat
X. cricarensis	single puddle	single puddle	Data Deficient (DD)	CR B1ab(iii)	Known for a seasonal pond, anthropogenic pressure, exotic fish
X. pataxo	~10.0	346.071	Vulnerable (VU)	EN B1ab(iii,v)	Drastic decline, limited occurrence, habitat loss
X. izecksohni	247.021	157.496	Near Threatened (NT)	VU B1ab(iii)	Habitat fragmentation, anthropogenic pressures
X. myersi	14.646	6.653	Endangered (EN)	CR B1ab(iii)	Habitat loss, anthropogenic pressures, small isolated populations

Figure 18. *Xenurolebias pataxo*. Images in field aquarium. Specimen not preserved. Image: Bruno Pinheiro.

Figure 19. Flooded area adjacent to Itanhém river, Alcobaça municipality, Bahia. Image: Luisa Sarmento.

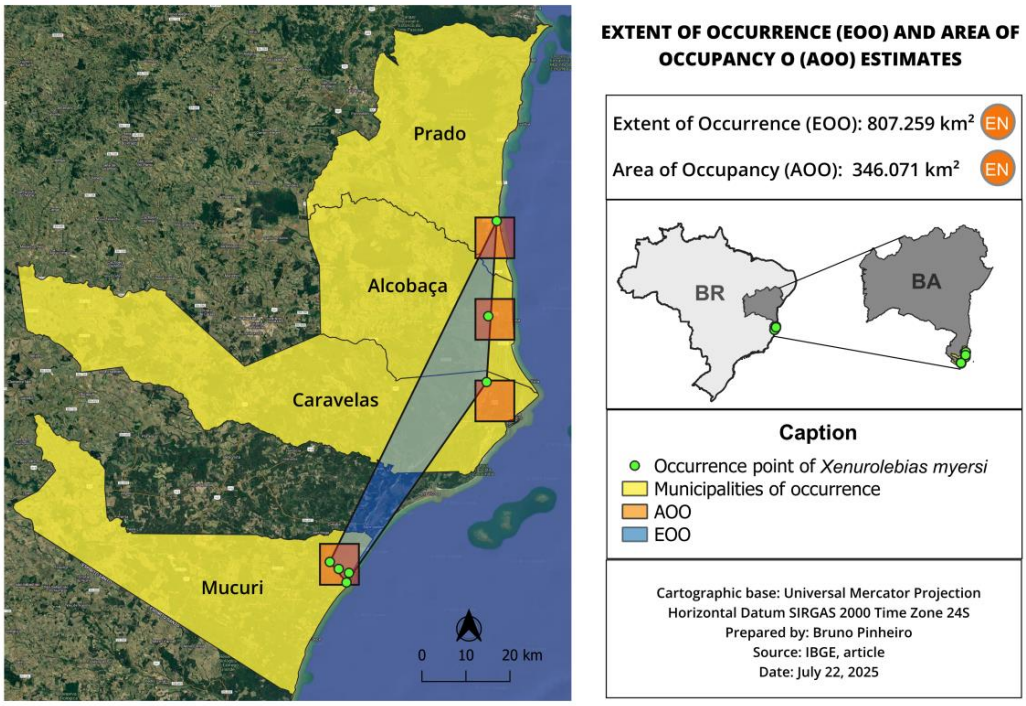

Figure 20. Estimates of EOO, cartographic area, and the number of locations for *Xenurolebias pataxo*.

4. Conclusions

The distribution of *Xenurolebias* species is linked to the flooded environments along the coastal tableland formations. Two of these species are threatened with extinction on Brazilian Red Lists (*X. myersi*-EN and *X. pataxo*-VU): one is Near-Threatened (*X. izecksohni*-NT); and one is Data-Deficient (*X. cricarensis*-DD). Most of these fish are on the Brazilian Red Lists [25], the state list of Espírito Santo [32], and the state list of Bahia [33].

Despite being impacted by changes in both terrestrial and aquatic systems, these coastal environments give shelter to an extraordinary diversity of taxa beyond annual killifish. Among these are the riverine freshwater fish threatened with extinction such as *Rachoviscus graciliceps*, *Ituglanis cahyensis*, *Mimagoniates sylvicola*, and also marine estuarine species as *Dormitator maculatus* and *Eleotris pisonis*. These fish find food, protection, and a nursery place in lowland rivulets, springs, and ponds.

The main impacts include eucalyptus and sugarcane monocultures, drainage of lowland areas (including temporary puddles), deforestation, and pollution. In the last ten years, impacts have been intense, especially for populations of *X. pataxo*, which had its habitat drastically reduced mainly due to habitat loss. Habitat degradation and loss due to human activities considerably impact temporary ponds, making them vulnerable and subject to great challenges regarding the viability of their populations. Some have a worrisome situation, as the population records are limited to fewer than three localities. Special attention should be given to two species of *Xenurolebias*: *X. cricarensis* known only from one locality and *X. tupinikin* known only from three very close localities. These fish inhabit the swamps of Cricaré River and Rio Riacho, respectively, out of protected areas. These localities are under pressure from occupation in their territories. In the Rio Riacho there is still the aggravating factor of the presence of garbage disposal in the vicinity of the puddle inhabited by these fish. There is an urgent need to reassess the conservation status of these two species. It is suggested that these fish can be considered as a Critically Endangered species in the next assessment of endangered fauna.

Riparian wetlands, where the *Xenurolebias* populations inhabit, vary in size, geography, and biological composition, and they function as transitional zones between land and

sea. The effective conservation of biodiversity in the riparian wetlands would ensure the connectivity between river corridors and floodplains, integrating aquatic and wetland habitats [19]. Negligence and habitat loss are among the threats that have rendered annual killifishes the most endangered fish in Brazil [34]. Ephemeral aquatic environments are often overlooked and undervalued, despite their high biodiversity [8,27]. Monitoring these fish populations in their respective habitats is even more necessary now because this directly implies the conservation of species and their environments. In this way, it is possible to understand the dynamics of the population, its habits, and behaviors. We are working for this purpose.

A collective possibility towards protecting most *Xenurolebias* populations in the coastal tablelands would be to work on the design of a mosaic of protected areas between Aracruz (ES) and Prado (BA).

The National Action Plan for the conservation of endangered rivulid fish, PAN Rivulids [26], highlights in its specific objective 3 the action of popularization of the species, through "disseminating knowledge about the focal species of rivulids, sensitizing society about the importance of wetlands for their conservation" [25,35]. Additionally, in the Espírito Santo state, it is a species benefited in the Capixaba-Gerais Territorial Action Plan—PAT Capixaba-Gerais [36].

Citizen science consists of the partnership between the residents of the place and the researchers in the discovery of novelties and collection of data for scientific research. The dialogue of knowledge, in which the community already knows about the place these fish live, and the protagonism of local actors, are sources of knowledge that were explored in *Xenurolebias myersi* [24,26,36]. Associating traditional knowledge with the technical-scientific nature can also enhance conservation actions for other species, through social participation in the construction of knowledge. We hope the present contribution may inspire future works on habitat and people interactions towards the survival of these species.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d17090644/s1, Table S1: Records of fish specimens listed for this study.

Author Contributions: All authors designed this study; B.P.G., L.M.S.-S., and R.F.M.-P. participated in fieldwork. R.F.M.-P. built the species vouchers list and checked species distribution ranges. L.M.S.-S. performed data curation and taxonomic validation. Statistical analysis was performed by G.R.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee for Animal Use of Federal University of Espírito Santo (CEUA-UFES); permit number: 008/23; approval date 30 August 2023, for studies involving field methods.

Data Availability Statement: Data is available in Supplementary Material Table S1.

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