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Two new species of *Atlantirivulus* (Cyprinodontiformes: Rivulidae) from Atlantic Forest biome, São Paulo State, Brazil

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Atlantirivulus santensis is currently considered as widely distributed along the coastal drainages of São Paulo State, Brazil. However, the morphological variation observed in this taxon suggests that it could represent a species complex. The objective of this study was to verify whether *A. santensis* may comprise more than one species using an integrative approach (DNA barcoding and morphology). We analyzed morphological traits and 76 partial sequences of the cytochrome c oxidase I region of the mitochondrial DNA of specimens identified as *A. santensis*, and additionally samples of *A. simplicis* and *A. ribeirensis*, for comparative purposes. The DNA barcoding analyses showed the existence of three clusters within *A. santensis*. The first clade consists of specimens from coastal rivers of the northern region of São Paulo State, the second from coastal rivers from the central region of São Paulo State and tributaries of the upper rio Tietê basin, and the third clade from specimens from coastal rivers from the southern of São Paulo State. Our data corroborates the existence of three *Atlantirivulus* species distributed along the coastal rivers of São Paulo State. *Atlantirivulus santensis* is redescribed, plus two new species, described herein.

Keywords: Conservation, DNA barcoding, Killifish, Rivulinae, Taxonomy.

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Atlantirivulus santensis é amplamente distribuída ao longo das drenagens costeiras do estado de São Paulo, Brasil. No entanto, a variação morfológica observada neste táxon sugere que ele poderia representar um complexo de espécies. O objetivo deste estudo foi verificar se *A. santensis* pode consistir em mais de uma espécie utilizando uma abordagem integrativa (DNA barcoding e morfologia). Foram analisadas características morfológicas e 76 sequências parciais da região do citocromo c oxidase I do DNA mitocondrial de espécimes identificados como *A. santensis*, e adicionalmente amostras de *A. simplicis* e *A. ribeirensis*, para fins comparativos. As análises de DNA barcoding mostraram a existência de três clusters dentro de *A. santensis*. O primeiro clado é composto por exemplares de rios litorâneos do norte do estado de São Paulo, o segundo por rios litorâneos da região central do estado de São Paulo e afluentes da bacia do alto rio Tietê, e o terceiro clado por exemplares de rios do litoral sul do estado de São Paulo. Nossos dados corroboram a existência de três espécies de *Atlantirivulus* distribuídas ao longo dos rios costeiros do estado de São Paulo. *Atlantirivulus santensis* é redescrita, e duas novas espécies são descritas.

Palavras-chave: Conservação, DNA barcoding, Killifish, Rivulinae, Taxonomia.

INTRODUCTION

Atlantirivulus Costa, 2008 is a genus of the family Rivulidae composed of 16 species, all occurring within the Atlantic Forest biome and distributed in coastal river systems from southern Bahia State southward to northern Rio Grande do Sul State, Brazil (Fricke *et al*., 2025): *A. depressus* (Costa, 1991), *A. enigmaticus* Volcan, Suárez, Severo-Neto, Amorim & Costa, 2024, *A. guanabarensis* Costa, 2014, *A. haraldsiolii* (Berkenkamp, 1984), *A. janeiroensis* (Costa, 1991), *A. jurubatibensis* (Costa, 2008a), *A. lazzarotoi* (Costa, 2007), *A. luelingi* (Seegers, 1984), *A. maricensis* Costa, 2014, *A. nudiventris* (Costa & Brasil, 1991), *A. paranaguensis* Costa, 2014, *A. ribeirensis* Costa, 2014, *A. riograndensis* (Costa & Lanés, 2009), *A. santensis* (Köhler, 1906), *A. simplicis* (Costa, 2004), and *A. unaensis* (Costa & De Luca, 2009).

Atlantirivulus species range between 25–45 mm in total length, with short and rounded fins, and are distinguished from all other members of the Rivulidae family, due to the unique pattern of the infraorbital neuromast series, arranged in a zigzag pattern, and a curved ventral process of the angulo-articular (Costa, 2008b). The species of this genus generally inhabit dense forest regions along the Brazilian coast, with exceptions like *A. riograndensis* and *A. jurubatibensis*, which are found in open areas lacking forest cover, in acidic shallow wetlands about 10 cm deep and at elevations below 100 m (Costa, 2011). Recently, *A. enigmaticus* was described by Volcan *et al*. (2024), collected in a region far from the Atlantic coast where other *Atlantirivulus* species occur. It was found in a shallow stream (~50 cm deep), in an ecotonal area between Atlantic Forest and Cerrado, near a cattle watering spot, with suppressed primary vegetation, approximately 700 km in a straight line from the coastal plains.

Atlantirivulus santensis, the first species of the genus to be described (Köhler, 1906) was redescribed by Costa (2008c), after 102 years, who considered it to occur along coastal streams from Peruíbe to Ubatuba, São Paulo State. The species is additionally recorded from a region on the plateau, close to the coast, in the São Paulo city. The type-locality of this species is not clear, but the descriptions presented by (Köhler, 1906) indicated that it is located occupying the central area of the species distribution, in the region known as Baixada Santista basin; officially known as UGRHI-07 (Water Resources Management Unit 07) and the region on the plateau, near São Paulo city.

In this study, we analysed specimens of *A. santensis* using DNA barcoding (COI) and morphological data to test the hypothesis that the species is broadly distributed along the coastal region of São Paulo State. The examination of recently collected material from surrounding coastal areas has resulted in the discovery of two additional species, previously unknown to science, which are formally described herein.

MATERIAL AND METHODS

Molecular analysis. 76 specimens of *Atlantirivulus* were used for the molecular analyses and are deposited and preserved in 95% ethanol in the collection of the Laboratório de Biologia de Peixes (LBP; Tab. **S1**). Total DNA was extracted from liver or muscle samples using the protocol by Ivanova *et al*. (2007). Partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene were amplified by polymerase chain reaction (PCR) using the primer set COI L6252–Asn (5'– AAG GCG GGG AAA GCC CCG GCA G –3') and H7271–COXI (5'– TCC TAT GTA GCC GAA TGG TTC TTT T –3') (Melo *et al*., 2011). The amplifications were performed in a total volume of 12.9 µL with 1.25 µL of 10X buffer (10 mM Tris-HCl), 0.375 µL MgCl (50 mM), 0.5 µL dNTPs (200 nM each), 0.25 µL of each primer (5 mM), 0.1 µL Platinum® Taq Polymerase (Invitrogen), 2 µL genomic DNA (12 ng) and 8,175 µL ddH2O. The PCR reaction consisted of the following programming: an initial step of 4 min at 94°C, 35 cycles of 30 sec at 94°C, 30 sec at 50–54°C and 1 min at 72°C and final extension of 10 min at 72°C. PCR products were visualized on 1% agarose gel and purified using ExoSap-IT® (USB Corporation, Cleveland, USA). These products were then submitted to sequencing reactions using the "BigDye® Terminator v. 3.1 Cycle Sequencing Ready Reaction" kit (Applied Biosystems, Austin, USA) and purified again by ethanol precipitation. Products were loaded onto an ABI 3130 DNA Analyzer automatic sequencer (Applied Biosystems). The DNA sequences obtained were analysed using the Geneious v. 4.2.2 (Kearse *et al*., 2012) and aligned with the Muscle algorithm (Edgar, 2004), implemented in the Geneious 4.2.2 program under the "default" parameters. The substitution saturation index (ISS) (Xia *et al*., 2003; Xia, Lemey, 2009) was estimated in the Dambe 5.3.38 program (Xia, Xie, 2001). Nucleotide variation, substitution patterns and genetic distances were estimated in MEGA v. 10 (Kumar *et al*., 2016).

Phylogenetics analysis and lineage delimitation. A Maximum Likelihood (ML) and Neighbor-Joining (NJ) tree were generated with 1000 bootstrap replicates (bs), using the T92 model (Tamura 3 parameters – Tamura, 1992) + G in MEGA v. 10.

Lineage delimitation analyses were performed using Assemble Species by Automatic Partitioning - ASAP (Puillandre *et al*., 2021) and Poisson Tree Processes - PTP (Zhang *et al*., 2013) methods. ASAP was performed through the web server and Poisson Tree Process using the maximum-likelihood (ML) tree, 100,000 generations, and other parameters at default on the bPTP webserver.

Morphological analysis. Measurements were made point by point under a stereomicroscope with a digital caliper to the nearest 0.01 mm on the left side of the specimens, whenever possible, following (Costa, 1995, 2007). The measurements are expressed as percentages of standard length (SL), except for subunits of the head, which are recorded as percentages of head length (HL). In the description, counts of vertebrae and pleural ribs were obtained from cleared and stained specimens (c&s), prepared according to Taylor, Van Dyke (1985). The terminology of frontal scaling and cephalic neuromasts follows Costa (2006). For vertebral counts, the center of the caudal compound was counted as a single element. The osteological characteristics included in the description are those considered phylogenetically informative by recent studies on *Atlantirivulus* (Costa, 2006, 2008b, 2011).

RESULTS

Molecular analysis. Seventy-six sequences of *Atlantirivulus* were generated in this study, and one sequence of the outgroup taxon *Cynodonichthys tenuis* Meek, 1904 was obtained from (GenBank number EU751964) from Valdez-Moreno *et al*. (2009). The matrix obtained comprised 541 base pairs (bp), of which 455 bp were conserved and 85 bp were variable. The nucleotide frequency was 34.8% thymine, 24.3% cytosine, 24.9% adenine and 15.9% guanine. The data did not show saturation considering that the Iss index did not indicate any saturation in transitions and transversions, in asymmetric (Iss. cAsym) and symmetric (Iss.cSym) topologies.

Phylogenetics analysis and lineage delimitation. The phylogenetics analysis ML and NJ recovered the same topology with high bootstrap values supporting each of the analyzed species and indicating the divergence of three phylogenetic clades within what was previously recognized as *A. santensis* (Figs. 1, **S2**). ASAP and bPTP identified five species, supporting *A. tupinambas* and *A. peruibensis* as new species (Fig. 1). The average interspecific divergence was 6% (± 1%) ranging from 3.4% between *A. santensis* and *A. peruibensis* to 12.4% between *A. tupinambas* and *A. simplicis* (Tab. 1). Intraspecific genetic variation ranged from 0 within *A. simplicis* and *A. ribeirensis* to 0.5% within *A. tupinambas* (Tab. 1).

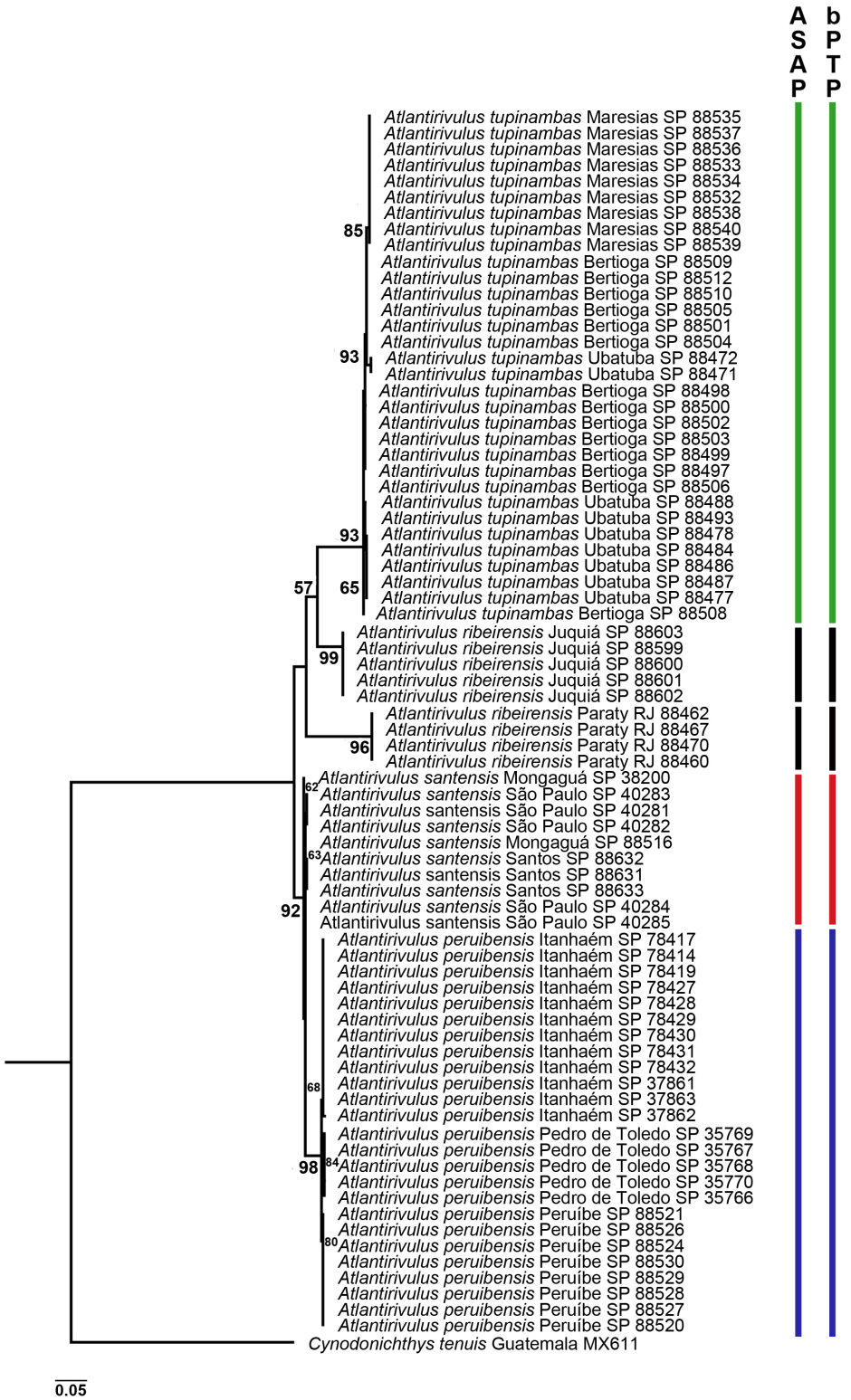

FIGURE 1 | Maximum likelihood tree of *Atlantirivulus* based on partial sequences of the gene cytochrome c oxidase subunit 1. Vertical bars represent the number of species delimited by ASAP and bPTP. Numbers near nodes represent bootstrap support. Codes after tip names represent voucher numbers.

TABLE 1 | Pairwise K2P genetic distance among species of *Atlantirivulus* (below diagonal) and values of standard error (above diagonal). Numbers in bold represent intraespecific K2P genetic distance.

Species	1	2	3	4	5
1. *A. simplicis*	-	0.017	0.017	0.016	0.017
2. *A. ribeirensis*	0.109	-	0.013	0.013	0.013
3. *A. tupinambas*	0.124	0.079	**0.005**	0.013	0.014
4. *A. peruibensis*	0.101	0.076	0.092	**0.004**	0.008
5. *A. santensis*	0.105	0.078	0.086	0.034	**0.002**

Atlantirivulus tupinambas, new species

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(Figs. 2–3; Tab. 2)

Holotype. LBP 34196, male, 31.4 mm SL, Brazil, São Paulo, Bertioga municipality, stream near Guaratuba beach, between the rio Itapanhaú and rio Guaratuba, 23°45'33.7"S 45°55'24.6"W, 25 Sep 2017, E. V. Ywamoto.

Paratypes. All from Brazil, São Paulo State: LBP 25426, 5 males (20.8–27.1 mm SL), 5 females (28.7–41.0 mm SL), 1 c&s; ZUEC 17918, 2 males (20.9–37.2 mm SL), 3 females (22.9–35.8 mm SL), same data as holotype. LBP 25432, 1 male (24.7 mm SL), 2 females (16.9–22.8 mm SL), São Sebastião, stream near Maresias beach, 23°47'29.9"S 45°33'15.4"W, 29 Jan 2018, E. V. Ywamoto. LBP 25425, 4 males (28.4–38.6 mm SL), Ubatuba, 23°30'00.1"S 45°07'37.4"W, 29 Jan 2018, E. V. Ywamoto. LBP 14301, 1 male (35.0 mm SL), Bertioga, 23°47'37.3"S 46°04'04.5"W, 29 Jun 2011, G. J. C. Silva, J. M. Henriques, J. C. P. Alves, B. Belo & R. Devidé. LBP 14339, 1 male (30.1 mm SL), 3 females (28.6–41.4 mm SL), São Sebastião, 23°45'31.8"S 45°41'05.4"W, 30 Jun 2011, G. J. C. Silva, J. M. Henriques, J. C. P. Alves, B. Belo & R. Devidé.

Diagnosis. *Atlantirivulus tupinambas* is distinguished from all other species of *Atlantirivulus* by a combination of color pattern features, *i.e.*, dark yellow ventral portion of head and venter (*vs.* venter white or pale yellow), light brown iris, with greenish-blue outline at the back (*vs.* dorsal region and inner portion of iris metallic light brown; second posterior third of iris metallic bluish green in *A. enigmaticus*); caudal-fin greenish-yellow, with a dark gray to black distal margin, it has dark pigmentation along the rays and dark gray punctuations that are concentrated in the basal and upper portion of the fin (*vs.* light yellow in *A. nudiventris*; dark red to black with broad dark to black margin and broad bright greenish yellow in *A. luelingi*; pale yellow with reddish brown dorsal and ventral marginal stripes in *A. riograndensis* and *A. haraldsiolii*; yellow with small gray spots on basal and dorsal portion in *A. simplices*; greenish-yellow in *A. janeiroensis*; yellow with horizontal dark stripes in *A. depressus*, pale yellow, often lighter on sub-dorsal portion; narrow reddish brown stripe on dorsal and ventral margins in *A. guanabarensis*; pale yellow, with broad ventral and narrow dorsal black margin, ventral

FIGURE 2 | *Atlantirivulus tupinambas*, LBP 34196, holotype, male, 31.4 mm SL, Brazil, São Paulo, Bertioga, stream at Guaratuba beach.

FIGURE 3 | *Atlantirivulus tupinambas*, LBP 25426, paratype, female, 31.7 mm SL, Brazil, São Paulo, Bertioga, stream at Guaratuba beach.

margin twice or thrice wider than dorsal margin *A. paranaguensis*; pale greenish yellow, sub-dorsal and sub-ventral portions bright yellow, dorsal and marginal region diffuse orangish grey, posterior marginal portion diffuse pale greyish yellow in *A. maricensis*; light yellow with board dark reddish dorsal and ventral margins in *A. jurubatibensis*; brown in *A. unaensis*; pale yellow with light blue dots in *A. lazzarotoi*; pale yellow, with narrow dark grey stripe along whole fin margin in *A. ribeirensis*; pale greyish green with light blue iridescence in the middle portion, with broad ventral and dorsal black margins with about equal width in *A. enigmaticus*), pelvic-fin blue with narrow anterior margin yellow (*vs*. pelvic-fin hyaline yellow, pale greyish green, in *A. enigmaticus*, dark greenish yellow in *A. luelingi*), in males.

It is distinguished from *A. nudiventris* by the presence of a pelvic-fin and pelvic girdle (*vs*. absence), in males and females, lower body depth (17.3–20.8% *vs*. 22.8% SL), in males. It is distinguished from *A. depressus* by a lower caudal peduncle depth (11.6–15.2% *vs*. 16% SL), in males. It is distinguished from *A. simplicis* by a lower pelvic-fin length (4.2–8.0% *vs*. 8.1–10.1% SL); dorsal-fin origin at a vertical through the 11th anal-fin ray (*vs*. 9th–10th), anal-fin origin at a vertical through the (17th–18th vertebrae *vs*. 14th–15th), fewer pelvic-fin rays 5 (*vs*. 6), and fewer pectoral-fin rays 13 (*vs*. 14), in males. It is distinguished from *A. jurubatibensis* by a lower pelvic-fin length (4.2–8.0% *vs*. 9.9–10.1% SL), a greater number of vertebrae (33–34 *vs*. 31–32), in males. It is distinguished from *A. maricensis* by the absence of small red spots on the basal and posterior portions of the dorsal fin (vs. red spots presence), in males. It is distinguished from *A. unaensis* by the anterior portion of the trunk deeper than wider (*vs*. wider than deeper) in males and females, lower number of pelvic-fin rays 5 (*vs*. 6), in males. It is distinguished from *A. janeiroensis* by a lower caudal-fin length (21.0–30.0% *vs*. 33.5%–40.1% SL) in males, (22.7–28.4% *vs*. 37.7–40.1% SL), in females. It is distinguished from *A. lazzarotoi* by the color pattern of the yellow anal fin, with a light blue basal portion, with distal margin dark gray to black (*vs*. orange yellow to reddish orange on distal half, with distal narrow black margin), in males. It is distinguished from *A. luelingi* by a lower body depth (17.3–20.8% *vs*. 21.1–23.0% SL), shorter pelvic-fin length (4.2–8.0% *vs*. 8.5–9.8% SL), fewer pelvic-fin rays 5 (*vs*. 6), a greater number of vertebrae (33–34 *vs*. 31–32), and the absence of multiple contact organs per flank scale (*vs*. presence), in males. It is distinguished from *A. enigmaticus* by a continuous lateral line (*vs*. lateral line divided into two sections, each section interrupted by scales without neuromast), in males. It is distinguished from *A. haraldsiolii* by fewer pectoral-fin rays 13 (*vs*. 14), a lower number of scales in the longitudinal series (33–34 *vs*. 35–36), a greater number of scales around the caudal peduncle 16 (*vs*. 14), fewer pelvic-fin rays (5 *vs*. 6), and absence of multiple contact organs per flank scale (*vs*. presence), in males. It is distinguished from *A. ribeirensis* by the anal-fin origin at a vertical through the (17th–18th vertebrae *vs*. 14th–16th), in males. It is distinguished from *A. guanabarensis* by the presence of triangular light blue spots on the flank (*vs*. absent), in males, not reaching pre-opercular series of neuromasts (*vs*. melanophore patch on the post orbital region extending ventrally to the pre-opercular series of neuromasts), in males and females. It is distinguished from *A. paranaguensis* by a shorter anal-fin length (18.1–19.2% *vs*. 21.8–23.7% SL), a shorter caudal-fin length (27.2–28.4% *vs*. 38.0–39.5% SL), a shorter pelvic-fin length (4.2–4.9% *vs*. 7.8–8.8% SL), in males. It is distinguished from *A. riograndensis* by a lower caudal penducle depth (11.5–12.7% *vs*. 13.8–15.4% SL), a greater number of scales in the longitudinal series (33–34 *vs*. 31–32), dorsal–fin origin at vertical through anal-fin ray 11th (*vs*. 9th–10th), dorsal-fin origin at vertical through vertebra (24th–25th *vs*. 21th–23th), in males.

Description. The largest male specimen examined measured 43.1 mm SL; the largest female measured 32.3 mm SL (Tab. 2). Dorsal and ventral profiles slightly convex from snout to posterior end of dorsal bases and anal fin, approximately in a straight line at caudal peduncle. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth vertically in front of pelvic-fin base. Short jaws, blunt snout. Absent multiple contact organs per flank scale in males.

TABLE 2 | Morphometric and meristic data of *Atlantirivulus tupinambas*. N = number of specimens.

	H Male	Males n = 14	Females n = 6
Standard length (mm)	31.4	23.4–43.1	19.3–32.3
Percents of standard length			
Body depth	18.6	17.3–21.7	19.2–21.7
Caudal peduncle depth	11.6	12.3–15.2	11.5–12.7
Predorsal length	76.3	72.4–80.9	74.6–80.4
Prepelvic length	51.9	45.2–67.6	48.6–56.4
Length of dorsal-fin base	7.8	6.2–11.2	7.7–11.4
Length of anal-fin base	18.6	17.9–23.1	18.1–19.2
Caudal-fin length	24.2	21.0–30.0	22.7–28.4
Pectoral-fin length	16.2	15.9–21.3	14.3–21.0
Pelvic-fin length	6	4.2–8.0	4.2–4.9
Head length	21.4	18.3–25.9	19.9–24.7
Percents of head length			
Head depth	71	52.8–84.2	65.7–75.5
Head width	80.3	75.8–95.4	77.5–93.5
Snout length	27.2	28.8–35,4	26.0–32.9
Lower jaw length	23.6	18.6–30.2	19.5–21.6
Eye diameter	32.2	31.6–39.4	29.8–35.5
Counts			
Dorsal-fin rays	7	7-9	7-8
Caudal-fin rays	31	28–33	28–30
Anal-fin rays	14	12–14	13–14
Pelvic-fin rays	5	5	5
Pectoral-fin rays	13	13	13

Dorsal and anal fins short, rounded, sometimes slightly pointed in males. Caudal fin oval, short. Pectoral fin rounded, posterior margin vertically reaching approximately 60% of length between pectoral fin and pelvic base. Pelvic fin small, oval, tip reaching between urogenital papilla and base of the 2nd anal-fin ray in males, and anus in females; pelvic-fin bases medially nearby. Dorsal–fin origin vertically at base of the 11th anal-fin ray, dorsal-fin origin at height of 24th–25th vertebrae, anal-fin origin at height of 17th–18th vertebrae. Dorsal-fin rays 7–9; anal–fin rays 12–14; caudal-fin rays 28–33; pectoral-fin rays 13; pelvic-fin rays 5.

Scales small and cycloid. Body and head entirely scaled, except on anterior ventral surface of t head. Body scale extending over 25% of caudal-fin base; no scales on bases of dorsal and anal fins. E-patterned frontal squamation; E-scales not overlapping medially. Longitudinal series of scales 33–34; transverse series of scales 8; scale rows around caudal peduncle 16.

Cephalic neuromasts: supraorbital 3 + 3, parietal 1, rostral anterior 1, rostral posterior 1, infraorbital 1 + 18–22 + 1, preorbital 1, otic 1, postoptic 2, supratemporal 1, median opercular 1, ventral opercular 1, preopercular 2 + 4, mandibular 3 + 1, lateral mandibular 1, paramandibular 1. Continuous lateral line, anterior section between head and vertical just anterior to end of pectoral fin, posterior section in longitudinal series just below anterior section, between vertical through end of pectoral fin and middle portion of caudal-fin base. Lateral line without neuromast by scale; 2 neuromasts at base of caudal fin. Dermosphenotic present. Ventral post temporal process absents. Total vertebrae 33–34.

Coloration in alcohol. Males. Body ground color orange, on the head to caudal peduncle. Venter whitish. Dorsum dorsal-fin grey. Anal-fin grey with black bar in inferior region. Caudal-fin light beige with darker base. Pelvic and pectoral fins light beige.

Females. Body ground color beige, from post-opercular region to caudal peduncle. Dorsum dark beige. Head beige with dark beige in opercular region. Dorsal, anal, pectoral and pelvic fins light beige. Caudal-fin light beige with two black bars in superior and inferior region.

Coloration in life. Males. Lateral of body light purple-brown with alternating yellowish-green oblique irregular bars and pale pink oblique bars. Dorsum light brown. Ventral portion of head and ventral area dark yellow. Side of head light brown to light gray above horizontal line across ventral margin of orbit, light yellow below eye; opercular region greenish-yellow; light gray jaw; light brown iris, with greenish-blue outline at the back; dorsal-fin light green, often with three or four narrow oblique pale brown stripes, with light blue basal portion; anal-fin yellow, basal portion light blue, with distal margin dark gray to black; caudal-fin greenish yellow, with a dark gray to black band on the distal margin, pigmentation along the rays and dark gray as punctuations concentrated in the basal and upper portion of the fin; pectoral-fin light beige with darker base; pelvic-fin bluish with anterior margin yellow.

Females. Lateral of body light brownish, with pale gray and light blue dots, concentrated between the pelvic and caudal. Dorsum light brown. Ventral area pale yellow. Side of head light brown. Postorbital region dark brown, ventral portion of opercular pale gold, suborbital light yellow; pale grayish brown jaws; iris yellow-orange, with a bluish outline at its inferior-posterior portion; hyaline yellowish dorsal-fin with transverse rows of small dark gray spots; anal-fin yellow, with light blue basal portion; caudal-fin pale yellow to hyaline orange, slightly bluish at base, with transverse rows of dark brown dots; dark gray circular spot on the dorsal portion of the base of the caudal-fin, sometimes discreet; paired fins yellowish hyaline.

Geographical distribution. The species has been recorded in coastal streams across five locations within São Paulo State, Brazil: one site in the municipality of Bertioga, two in São Sebastião, and two in Ubatuba (Fig. 4). It occupies a variety of habitats, including areas impacted by human activity. Despite this apparent adaptability, the species is increasingly threatened by urban expansion and the consequent decline of native vegetation. Our findings highlight the need for further research to refine the understanding of its geographic distribution, monitor population trends, and deepen knowledge of its ecological and biological traits.

Ecological notes. Occurs in a variety of aquatic environments, including small forest pools, stream banks, and water channels, in both still and slow-flowing waters. Found in habitats ranging from crystal-clear to dark-coloured, still or slow-flowing waters.

Etymology. In reference to the Tupinambás indigenous tribe that inhabited the region. A noun in apposition, unchanged latinized eponym. The Tupinambás were an Indigenous Brazilian people belonging to the Tupi-Guarani linguistic family. Before the arrival of the Portuguese, they inhabited the Brazilian coast, primarily in the Northeast,

Southeast, and South regions. They are noted for their rich cultural heritage, which included warfare, anthropophagic rituals, and social organization within communal dwellings known as malocas.

Conservation status. *Atlantirivulus tupinambas* has a wide distribution on the northern coast of the state of São Paulo, between the municipalities of Bertioga and Ubatuba, in a coastal plain with approximately 140 km of extension (Fig. 4). It occurs in various habitats, which include adapting to altered areas, impacted by anthropogenic activities, particularly the advance of urbanization and the consequent decrease in native vegetation. In light of the need for a more comprehensive analysis, we recommend classifying the species as Data Deficient (DD), indicating that there is currently insufficient information to accurately evaluate its risk of extinction, following criterion of the International Union for Conservation of Nature (IUCN Standards and Petitions Subcommittee, 2024).

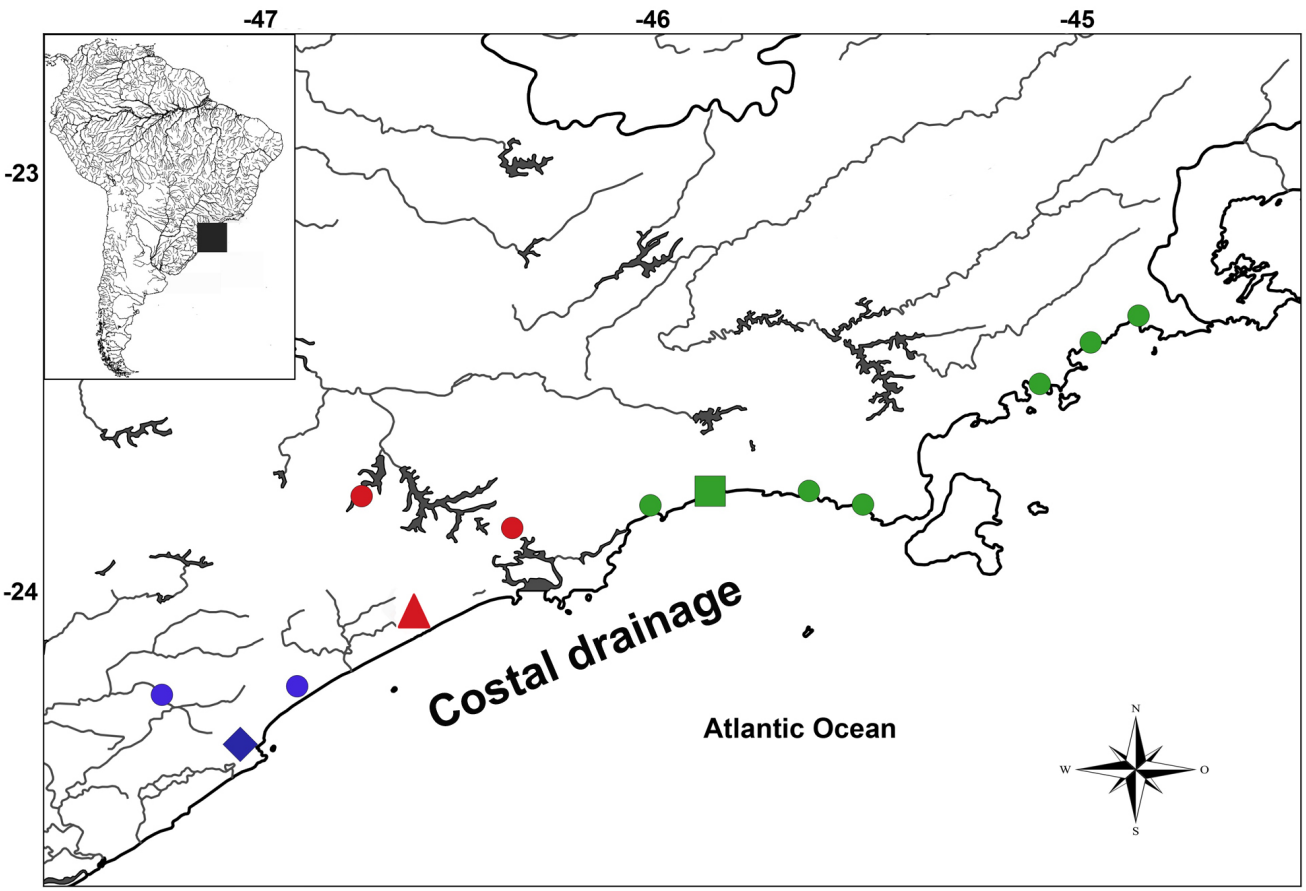

FIGURE 4 | Map of São Paulo State coast, Brazil with the register of *A. tupinambas* (green square type-locality, green circles other samples localities), *A. santensis* (red triangle type-locality, red circles other samples localities), and *A. peruibensis* (blue diamond type-locality, blue circles other samples localities).

Atlantirivulus peruibensis, new species

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(Figs. 5-6; Tab. 3)

Holotype. LBP 34197, male 32.6 mm SL, Brazil, São Paulo, Peruíbe municipality, stream tributary of rio Guaraú, at the Guaraúna road, 24°22'17.7"S 47°03'32.1"W, 29 Jan 2018, E. V. Ywamoto, F. G. Paim, R. Devidé & L. Soares.

Paratypes. All from Brazil, São Paulo State: LBP 6826, 2 females (53.0–55.2 mm SL), Itanhaém, 24°10'53.3"S 46°50'33.8"W, 3 Sep 2008, C. Oliveira, M. Alexandrou, J. C. P. Alves & J. M. Henriques. LBP 8128, 4 males (36.2–42.9 mm SL), 4 females (32.0–47.8 mm SL), 1 c&s, Itanhaém, 24°13'55.0"S 46°55'14.9"W, 27 May 2009, R. Devidé, R. Britzke, G. J. C. Sila & V. A. Tagliacollo. LBP 8191, 6 males (23.8–36.7 mm SL), 4 females (16.9–40.7 mm SL), Itanhaém, 24°13'54.9"S 46°55'15.1"W, 13 Aug 2009, R. Devidé, R. Britzke, G. J. C. Sila & V. A. Tagliacollo. LBP 31528, 7 males (18.6–38.7 mm SL), 11 females (16.8–29.1 mm SL), Itanhaém, ditch 2.8 km from road BR-101, 24°13'08.9"S 46°55'24.7"W, 18 Sep 2011, D. T. B. Nielsen & A. B. Caldeira Jr. ZUEC 17919, 2 males (26.8–28.9 mm SL), 3 females (25.5–31.3 mm SL), Itanhaém, ditch 2.8 km from road BR-101, 24°13'08.9"S 46°55'24.7"W, 18 Sep 2011, D. T. B. Nielsen & A. B. Caldeira Jr.

FIGURE 5 | *Atlantirivulus peruibensis*, LBP 34197, holotype, male, 32.6 mm SL, Brazil, São Paulo, Peruíbe, stream tributary of rio Guaraú, at the Guaraúna road.

FIGURE 6 | *Atlantirivulus peruibensis*, LBP 6826, paratype, female, 19.9 mm SL, Brazil, São Paulo, Peruíbe, stream tributary of rio Guaraú, at the Guaraúna road.

Diagnosis. *Atlantirivulus peruibensis* is distinguished from all other species of *Atlantirivulus* by a dorsal-fin origin at vertical through vertebra 25th–26th (*vs*. 20th–23rd); anal-fin origin at vertical through vertebra 18th–19th (*vs*. 14th–17th), except *A. tupinambas*; shorter caudal-fin length (23.0–31.3% *vs*. 33.0–43.7% SL) except *A. ribeirensis* and *A. paranaguensis* and greater number of vertebrae (35 *vs*. 34), except *A. paranaguensis*, in males. It is distinguished from *A. depressus* by a lower caudal peduncle depth (10.6–14.6% *vs*. 16.0% SL), in males, a dorsal-fin origin at the vertical through the 11th anal-fin ray (*vs*. 9th), a greater number of caudal-fin rays (30–32 *vs*. 27–28), in males. A greater number of longitudinal series of scales (35 *vs*. 33–34), in males and females. It is distinguished from *A. simplicis* by the dorsal-fin origin at a vertical through the 11th anal fin ray (*vs*. 9th–10th), in males. It is distinguished from *A. jurubatibensis* by a shorter pelvic-fin length (5.1–9.2% *vs*. 9.9–10.1% SL), a greater number of pectoral-fin rays 14 (*vs*. 13), a greater number of caudal-fin rays (30–32 *vs*. 25–29), a greater number of pelvic fin rays (6 *vs*. 5), a greater number of pectoral-fin rays (14 *vs*. 13), and a greater number of transverse series of scales (8 *vs*. 7), in males. It is distinguished from *A. unaensis* by a greater number of caudal-fin rays (30–32 *vs*. 24–25), a greater number of transverse series of scales 8 (*vs*. 7), anterior portion of the trunk deeper than wider (*vs*. wider than deeper), in males. It is distinguished from *A. janeiroensis* by a lower caudal peduncle depth (10.6–14.6% *vs*. 16.0% SL), in males. It is distinguished from *A. janeiroensis* by a shorter caudal-fin length (23.0–31.3% *vs*. 33.5–40.1% SL) in males and (21.1–28.9% *vs*. 37.7–40.1% SL) in females, and by a greater number of transverse series of scales 8 (*vs*. 7), in males. It is distinguished from *A. lazzarotoi* by the color pattern of the anal-fin, greenish, with a blue basal portion and a dark gray to black distal margin (*vs*. orange-yellow to reddish orange on the distal half, with a narrow black distal margin); by jaw

TABLE 3 | Morphometric and meristic data of *Atlantirivulus peruibensis*. N = number of specimens.

	H Male	Males n = 19	Females n =24
Standard length (mm)	32.6	19.2–39.0	15.4–35.0
Percents of standard length			
Body depth	21.3	15.5–22.3	17.6-32.0
Caudal peduncle depth	13.6	10.6–14.6	9.9–14.0
Predorsal length	75.3	70.3–78.5	72.4–79.3
Prepelvic length	53.6	45.5–57.3	48.7-56.1
Length of dorsal-fin base	7.3	5.0–14.2	6.2–13.1
Length of anal-fin base	18.8	15.2–27.2	13.7–23.3
Caudal-fin length	20.9	23.0–31.3	21.1–28.9
Pectoral-fin length	16.7	15.2–22.7	13.7-22.0
Pelvic-fin length	5.9	5.1–9.2	5.1-5.7
Head length	23.6	19.8–28.5	23.4–28.1
Percents of head length			
Head depth	71.4	53.2-69.5	51.7–66.4
Head width	81.4	65.3-87.1	61.2-76.3
Snout length	26	24.2-27.1	14.4-19.1
Lower jaw length	20.5	15.8–23.2	16.5–21.6
Eye diameter	35.5	27.8–42	29.8–39.8
Counts			
Dorsal-fin rays	8	8	8
Caudal-fin rays	31	30–32	30
Anal-fin rays	14	13–14	14
Pelvic-fin rays	6	6	6
Pectoral-fin rays	14	14	14

coloration-yellow (*vs*. red); and iris color-brown (*vs*. blue), in males. It is distinguished from *A. enigmaticus* by the flank coloration, light brown with alternating pale orange and pale pink oblique bars, sometimes barely visible and a light brown back (*vs*. purplish metallic green with brownish-red dots scattered along the body, forming 4–5 non-continuous horizontal rows), in males. It is distinguished from *A. riograndensis* by a greater number of pelvic-fin rays 6 (*vs*. 5), a dorsal-fin origin at the vertical through the 11th anal-fin ray (*vs*. 9th–10th), in males. The molecular analyses provide additional support towards the recognition of the new species.

Description. The largest male specimen examined measured 39.0 mm SL; the largest female measured 35.0 mm SL (Tab. 3). Dorsal and ventral profiles weakly convex from snout to posterior end of dorsal bases and anal fin, approximately in a straight line on caudal peduncle. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth vertically in front of pelvic-fin base. Short jaws, blunt snout.

Dorsal and anal fins short, tip rounded, sometimes slightly pointed in males. Caudal fin oval, short. Pectoral-fin rounded, posterior margin reaching vertically around 60% of length between pectoral-fin and base of pelvis. Pelvic-fin small, oval, tip reaching between urogenital papilla and base of 2nd anal-fin ray in males, and anus in females; pelvic-fin bases medially nearby, 22% of females absent pelvic fin, 1 female has only one

pelvic fin. Dorsal-fin origin vertically at base of the 11th anal-fin ray, dorsal-fin origin at height of 25th–26th vertebra, anal-fin origin at height of 18th–19th vertebra. Dorsal-fin rays 8; anal-fin rays 12–14; caudal-fin rays 30–32; pectoral-fin rays 14; pelvic-fin rays 6.

Scales small and cycloid. Body and head entirely scaled, except on anterior ventral surface of head. Body scale extending over 25% of caudal-fin base; no scales on bases of dorsal and anal fins. E-patterned frontal sqamation; E-Scales not overlapping medially. Longitudinal series of scales 35; transverse series of scales 8; scales row around caudal peduncle 16. One or two small scale-contact organs from ventral region of mid-flank in males; without contact organs in the fins.

Cephalic neuromasts: supraorbital 3 + 3, parietal 1, rostral anterior 1, rostral posterior 1, infraorbital 1 + 18–23 + 1, preorbital 2, otic 1, postoptic 2, supratemporal 1, median opercular 1, ventral opercular 1, preopercular 2 + 4, mandibular 3 + 1, lateral mandibular 1, paramandibular 1. Lateral line divided into 2 sections, anterior section between head and vertical just anterior to end of pectoral fin, posterior section in longitudinal series just below anterior section, between vertical through end of pectoral fin and middle portion of base of the caudal-fin; 1 neuromast per lateral line scale; 2 neuromasts at base of caudal-fin. Six branchiostegal rays. Dermosphenotic present. Ventral posttemporal process absent. Total vertebrae 35.

Coloration in alcohol. Males. Body ground color light brown, on head to caudal peduncle, venter whitish. Dorsum brown. Dorsal, anal and caudal fins hyaline brown. Pelvic and pectoral fins hyaline.

Females. Body ground light brown, opercular region to caudal peduncle. Dorsum brown. Dorsal and anal fins hyaline brown, pelvic and pectoral fins hyaline. Caudal-fin hyaline brown with two black bars in superior and inferior region; dark spot on dorsal base of caudal-fin.

Coloration in life. Males. Side of the body light brown with alternating pale orange oblique bars and pale pink oblique bars, sometimes barely visible; light brown back. Venter light yellow, with stronger yellow on the jaw. Side of head light brown, light yellow below the eye. Side of head light brown, light yellow below eye, slightly greenish yellow in ventral portion and red brownish upper portion of opercular region. Jaw light brown upper and yellow lower. Iris brown. Dorsal-fin gray, often with bright yellow brown spots; anal–fin greenish, basal portion light blue, with distal margin dark gray to black. Caudal-fin dark yellowish gray; dark gray to black stripe on dorsal and ventral margins of fin, both short making them inconspicuous. Pectoral-fin hyaline yellowish. Pelvic-fin hyaline yellow with bluish tip.

Females. Side of body light brownish, with light yellow spots; light golden brown back; whitish belly, with pale yellow in the head region; side of head brown, post-orbital region dark brown, ventral portion of opercular golden yellow, suborbital light yellow; pale grayish brown jaws; orange-brown iris; slightly bluish-green hyaline dorsal-fin with transverse rows of small dark gray spots; anal-fin yellowish green, with basal portion light blue and distal portion grayish brown; yellowish hyaline caudal-fin, slightly bluish at the base, with transverse rows of dark brown dots; dark gray circular spot on the dorsal portion of the base of the caudal fin, sometimes inconspicuous; hyaline pectoral fins; pelvic fins hyaline, slightly bluish and yellowish in the anterior portion.

Geographical distribution. *Atlantirivulus peruibensis* is distributed along the southern coast of São Paulo State, specifically within the municipalities of Itanhaém and Mongaguá (Fig. 4). The species inhabits a range of environments, including anthropogenically altered areas. Nevertheless, it faces significant pressures from ongoing urban expansion and the resulting loss of native vegetation. Based on our findings, further research is required to delineate the species' distributional boundaries, assess population dynamics, and better understand its ecological and biological characteristics.

Etymology. Peruíbe, in reference to the municipality where the terra typica of the species is located. Noun in apposition, unchanged latinized toponym.

Conservation status. The geographic distribution includes urban areas in the city of Peruíbe as well as other municipalities, such as Itanhaém and Pedro de Toledo, where many aquatic habitats have been destroyed over recent decades. Given the necessity for a more thorough analysis, we propose classifying the species as Data Deficient (DD), in accordance with the criteria set by the International Union for Conservation of Nature (IUCN Standards and Petitions Subcommittee, 2024). This designation reflects the current lack of sufficient data required to reliably assess the species extinction risk.

Atlantirivulus santensis Köhler, 1906

(Figs. 7–8; Tab. 4)

Rivulus elegans Steindachner var. *santensis* Köhler, 1906:408 (original combination and description; type-locality: Santos [São Paulo State, southeastern Brazil]; no type designated). —Huber, 1992:396 (redescription; from western surroundings of Santos, near Pedro Taques, São Paulo, Brazil; designated neotype MNHN1991-6856).

Rivulus urophthalmus (non *R. urophthalmus* Günther, 1866). —Henn, 1916:111 (misidentification Raiz da Serra, Cubatão and Iguape, São Paulo).

Rivulus rachovii Ahl, 1925:109 (type-locality: Para [locality incorrect, *e.g.*, Seegers, 1984]; lectotype: ZMB 31316, designated by Seegers, 1984).

Diagnosis. *Atlantirivulus santensis* is distinguished from all other species of *Atlantirivulus* by the combination of color pattern features, *i.e.*, light yellow-brown iris (*vs*. light yellowish to greenish brown in *A. haraldsiolii*; light yellowish with bright green margins in *A. luelingi*; light yellow in *A. simplicis* and *A. jurubatibensis*; brown with dark green margin in *A. maricensis*, *A. ribeirensis*, *A. paranaguensis*, and *A. guanabarensis*; light greenish yellow in *A. riograndensis*; brown in *A. nudiventris*; light blue in *A. lazzardoi*; yellow in *A. janeiroensis*; dorsal region and inner portion of iris metallic light brown, second posterior third of iris metallic bluish green in *A. enigmaticus*), caudal-fin greenish-yellow, with a dark stripe on the lower edge about 2–3 times wider than the upper edge (*vs*. light yellow in *A. nudiventris*; dark red to black with broad dark to black margin and broad bright greenish yellow in *A. luelingi*; pale yellow with reddish brown dorsal and ventral marginal stripes in *A. riograndensis* and *A. haraldsiolii*; yellow with small gray spots on basal and dorsal portion in *A. simplices*; greenish-yellow in *A. janeiroensis*; yellow with horizontal dark stripes in *A. depressus*; pale yellow, often lighter

FIGURE 7 | *Atlantirivulus santensis*, LBP 25428, male, 31.2 mm SL, Monguaguá, São Paulo, Brazil.

FIGURE 8 | *Atlantirivulus santensis*, LBP 35058, female, 23.7 mm SL, Monguaguá, São Paulo, Brazil.

on sub-dorsal portion; narrow reddish brown stripe on dorsal and ventral margins in *A. guanabarensis*; pale yellow, with broad ventral and narrow dorsal black margin, ventral margin 2–3 times wider than dorsal margin *A. paranaguensis*; pale greenish yellow, sub-dorsal and sub-ventral portions bright yellow, dorsal and marginal region diffuse orangish grey, posterior marginal portion diffuse pale greyish-yellow in *A. maricensis*; light yellow with board dark reddish dorsal and ventral margins in *A. jurubatibensis*; brown in *A. unaensis*; pale yellow with light blue dots in *A. lazzarotoi*; pale yellow, with narrow dark grey stripe along whole fin margin in *A. ribeirensis;* purplish metallic green and with brownish red dots scattered along of the body forming 4–5 non-continuous horizontal rows, in *A. enigmaticus*), in males. It is distinguished from *A. nudiventris* by

the presence of a pelvic-fin and pelvic girdle (*vs*. absence), in males and females; by a lower body depth (15.9–20.9% *vs*. 22.8% SL), in males. It is distinguished from *A. depressus* by a lower caudal peduncle depth (11.3–15.1% *vs*. 16% SL), a greater number of pelvic-fin rays 6 (*vs*. 4–5), and a dorsal-fin origin at the vertical through the 11th anal-fin ray (*vs*. 9th), in males. It is distinguished from *A. simplicis* by a shorter pelvic-fin length (4.3–6.1% *vs*. 8.1–10.1% SL), shorter caudal-fin length (23.7–32.8% *vs*. 37.2–40.0% SL), fewer caudal-fin rays (28–31 *vs*. 32–34), a greater number of vertebrae 35 (*vs*. 33), in males. It is distinguished from *A. jurubatibensis* by a shorter pelvic-fin length (4.3–6.1% *vs*. 9.9–10.1% SL), a greater number of vertebrae 34 (*vs*. 31–32), and a greater number of transverse series of scales 8 (*vs*. 7), in males. It is distinguished from *A. luelingi* and *A. haraldsiolii* by the absence of multiple contact organs per flank scale (*vs*. presence), in males. It is distinguished from *A. unaensis* by having the anterior portion of the trunk deeper than wider (*vs*. wider than deeper), in males. It is distinguished from *A. janeiroensis* by a lower caudal penducle depth (11.3–15.1% *vs*. 16% SL), shorter caudal-fin length (23.7–32.8% *vs*. 33.5–40.1% SL), in males; and (26.9–31.6% *vs*. 37.7–40.1% SL), in females, a greater number of transverse series of scales 8 (*vs*. 7), in males. It is distinguished from *A. lazzarotoi* by a lower number caudal-fin rays (28–31 *vs*. 32–34), a greater number of transverse series of scales 8 (*vs*. 7), in males. It is distinguished from *A. riograndensis* by a shorter dorsal-fin length (7.2–9.3% *vs*. 9.6–11.3% SL), a shorter caudal-fin length (23.7–32.8% *vs*. 37.7–40.0% SL), a greater number of pelvic-fin rays 6 (*vs*. 5), dorsal-fin origin at vertical through the 24th–25th vertebra (*vs*. 21st–23rd), and dorsal-fin origin at vertical through the 11th anal-fin ray (*vs*. 9th–10th), in males.

Description. Largest male examined 54.6 mm SL, largest female examined 58.6 mm SL (Tab. 4). Dorsal and ventral profiles weakly convex from snout to posterior end of dorsal and anal-fin bases, approximately in straight line at caudal peduncle. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth vertically in front of pelvic-fin base. Short jaws, snout slightly pointed. Absent multiple contact organs per flank scale in males

Dorsal and anal fins short, rounded, sometimes slightly pointed in males. Caudal-fin usually sub-truncate, sometimes elliptical. Pectoral-fin rounded, posterior margin vertically reaching approximately 60% of length between pectoral-fin and pelvic base. Pelvic-fin small, oval, tip reaching between urogenital papilla and base of urogenital area in males and females; pelvic-fin bases medially nearby. Dorsal-fin origin vertically at base of the 11th anal-fin ray, dorsal-fin origin at height of 24th–25th vertebra, anal-fin origin at height of 15th–16th vertebra. Dorsal-fin rays 8; anal-fin rays 14; caudal-fin rays 28–31; pectoral-fin rays 12; pelvic fin rays 6.

Scales small and cycloid. Body and head entirely scaled, except on anterior ventral surface of head. Body scale extending over 25% of caudal-fin base; no scales on bases of dorsal and anal fins. E-patterned frontal suamation; E-scales not overlapping medially. Longitudinal series of scales 33–34; transverse series of scales 8; scales rows around caudal peduncle 16.

Cephalic neuromasts: supraorbital 3 + 3, parietal 1, rostral anterior 1, rostral posterior 1, infraorbital 1 + 18–22 + 1, preorbital 2, otic 1, post-optic 2, supratemporal 1, median opercular 1, ventral opercular 1–2, preopercular 2 + 4, mandibular 4 + 2, lateral mandibular 1, paramandibular 1. Lateral line divided into 2 sections, anterior

section between head and vertical just anterior to end of pectoral fin, posterior section in longitudinal series just below anterior section, between vertical through end of pectoral–fin and middle portion of caudal-fin base; 1 neuromast per lateral line scale; 2 neuromasts at base of caudal fin. Dermosphenotic present. Ventral post temporal process absents. Total vertebrae 34.

Coloration in alcohol. Males. Body ground color black, on head to caudal peduncle, venter light brown, dorsal fin grey, anal fin grey with black bar in inferior region, anal fin light beige with darker base. Pelvic and pectoral fins light beige.

Females. Body ground beige color, opercular region to caudal peduncle. Dorsum dark beige. Head beige with dark beige in opercular region. Dorsal, anal, pectoral and pelvic fins light beige. Caudal-fin light beige with two black bars in superior and inferior region.

TABLE 4 | Morphometric and meristic data of *Atlantirivulus santensis*.

	Males n = 15	Females n = 13
Standard length (mm)	20.0–54.6	18.1–58.6
Percents of standard length		
Body depth	15.9–20.9	17.5–18.9
Caudal peduncle depth	11.3–15.1	9.4–12.1
Predorsal length	73.0–80.9	75.0–80.1
Prepelvic length	47.7–67.6	52.4–61.2
Length of dorsal-fin base	7.2–10.3	8.0–9.4
Length of anal-fin base	15.8–22.6	15.9–17.5
Caudal-fin length	23.7–32.8	26.8–31.6
Pectoral-fin length	15.4–22.6	15.5–19.6
Pelvic-fin length	4.3–6.1	4.1–5.7
Head length	21.5–25.4	24.2–27.7
Percents of head length		
Head depth	65.9–73.6	61.2–66.1
Head width	70.9–82.8	72.3–81.4
Snout length	25.1–28.3	22.9–25.3
Lower jaw length	16.9–21.8	16.7–20.3
Eye diameter	33.1–39.5	35.1–37.9
Counts		
Dorsal-fin rays	8	8
Caudal-fin rays	28–31	28–30
Anal-fin rays	14	14
Pelvic-fin rays	6	6
Pectoral-fin rays	12	12

Coloration in life. Males. Lateral of the body light purple-brown with narrow bright yellowish-green oblique bars barely visible. Dorsum light brown. Ventral portion of head and ventral area pale yellow. Side of head light brown above horizontal line across ventral margin of orbit, brown below eye; yellow and greenish opercular region; light gray mandibles; light yellow-brown iris, dorsal fin pale yellow, with narrow oblique pale brown stripes, distal area with bright yellow in in the first rays; anal fin bright yellow, basal portion bright green, with distal margin brown. Caudal-fin greenish-yellow, with a dark stripe on the lower edge about 2–3 times wider than the upper edge, pectoral fin hyaline yellowish, pelvic fin yellow.

Females. Lateral of the body gray-brown with light green and bright yellow dots. Dorsum light brown with brown stains. Ventral portion of opercular whitei, yellowish close to the jaw, pale grayish jaws; orange-brown iris. Dorsal fin hyaline with little bright yellow and dark gray dots; anal fin hyaline, with light blue basal dots; caudal fin hyaline yellow; black spot on the dorsal portion of the base of the caudal fin, with a smaller white spot on the side; paired yellowish hyaline fins.

Geographical distribution. The geographic distribution of the species *A. santensis* has undergone changes due to the evolving understanding of what is truly considered *A. santensis*. In its original description, Köhler, 1906 refers to the species from the type-locality: Santos, State of São Paulo, southeastern Brazil, with no type designated. In 1916, Henn misidentified specimens from Raiz da Serra, Cubatão, and Iguape, São Paulo. In 1925, there was a record of *Rivulus rachovii*, with the type-locality listed as Para (an incorrect locality, as noted by Seegers, 1984); the lectotype (ZMB 31316) was designated by Seegers in 1984. Later, in 1992, Huber established a neotype: MNHN 1991-6856, from the western surroundings of Santos, near Pedro Taques, São Paulo, Brazil.

In 1991, Costa defined the species' distribution using material deposited at MZUSP and UFRJ, with the southern limit in Paranaguá, Paraná, and the northern limit in Ubatuba, São Paulo. In the first redescription of *A. santensis*, Costa (2008) maintained the same distribution as defined in 1991. In 2014, Costa described *A. paranaguaensis* and *A. ribeirensis*, determining that the populations from Paranaguá, Paraná, were described as *A. paranaguaensis*, and those from the Ribeira River basin in São Paulo state were described as *A. ribeirensis*. In this work, with the description of *A. tupinambas* and *A. peruibensis*, and the discovery of a new population in the city of Embu, in the plateau region, the distribution of *A. santensis* is now recorded from coastal streams in the cities of Santos, Cubatão, Praia Grande, and Mongaguá, as well as the plateau region near São Paulo city.

Ecological notes. This species inhabits a wide range of aquatic environments, including small streams, forest pools, flooded areas, and both natural and modified marshes dominated by Taboa plants (*Typha domingensis*). Water conditions across these habitats vary, ranging from dark, acidic waters to lighter-colored waters with neutral pH. In some sites, high levels of eutrophication have also been observed.

Conservation status. *Atlantirivulus santensis* had a wide distribution on the coast of the State of São Paulo and in the metropolitan region of São Paulo city. Its distribution area was reduced, with new studies, where it was found that what was understood by

A. santensis was a complex of species formed by *A. ribeirensis*, *A. peruibensis*, *A. santensis* and *A. tupinambas*. Its distribution area was restricted to the metropolitan region of the city of São Paulo and the region known as Baixada Santista (Fig. 4). This area is one of the most environmentally impacted, due to urban expansion, reduction of native vegetation and pollution of water bodies. Its conservation status was considered Least Concern (LC) by Pavanelli *et al*. (2023). SALVE (Biodiversity Extinction Risk Assessment System), a system coordinated by ICMBIO with the most recent update on September 17, 2021, but considering our findings more studies are needed regarding the distribution limits, population trends and biology of *A. santensis*. It is important to highlight there are no conservation programs for this species in Brazil.

Material examined. Brazil, São Paulo: *Atlantirivulus santensis*: LBP 8211, 1 male (27.6 mm SL), Monguaguá, 24°04'57.6"S 46°37'11.0"W, 27 May 2009, R. Devidé *et al*. LBP 8733, 4 males (22.7–28 mm SL), São Paulo, 23°46'16.8"S 46°45'56.4"W, 1 Sep 2009, O. T. Oyakawa. LBP 25428, 1 male (31.2 mm SL), Monguaguá, 24°04'57.6"S 46°37'11.0"W, 25 Sep 2017, E. V. Ywamoto & G. Hollandres. LBP 34180, 3 males (24.5–27.3 mm SL), Cubatão, 23°50'54.0"S 46°24'06.5"W, 15 Jun 2018, E. V. Ywamoto & C. S. Souza. LBP 35058, 6 males (54.6–44.3mm SL), 13 females (59.5–38.4 mm SL), Embu Guaçu, 23°48'7.62"S 46°48'42.07"W, 26 Jun 2021, V. F. S. Souza.

DISCUSSION

We present two new species of the genus *Atlantirivulus*. With their introduction and the revised account of *A. santensis*, the genus now includes 18 valid species. One, *Atlantirivulus tupinambas*, is currently restricted to a coastal plain between the municipalities of Bertioga and Ubatuba in São Paulo State. The second, *A. peruibensis*, is likewise found within a limited zone encompassing Itanhaém and Mongaguá, São Paulo State. These additions reflect the distribution trend observed in most other species of *Atlantirivulus*, they are endemic to narrow ranges along coastal plains (Costa, 2011), making them particularly vulnerable to anthropogenic impacts.

Until the description of *A. ribeirensis* by Costa in 2014, all known species of *Atlantirivulus* were recorded from coastal plains. In that account, the holotype of *A. ribeirensis* was found on a plateau in the municipality of Juquiá, at approximately 125 m elevation. Subsequently, a population of *A. santensis* was discovered in Embu at 790 m elevation, and more recently *A. enigmaticus* was found on a plateau approximately 700 km inland, at about 300 m elevation in municipality of Amambaí, Mato Grosso do Sul State. These findings suggest that the genus' distribution is broader than previously assumed and not confined to coastal regions. Dedicated fieldwork focused on identifying aquatic habitats relevant to this genus is essential to better understand its actual distribution.

Costa (2014) observed that *A. guanabarensis*, *A. maricensis*, *A. paranaguensis*, and *A. ribeirensis* belong to the *A. santensis* species group, which is diagnosable by "the lateral line divided in two sections: the anterior one placed between the head and a vertical just anterior to the end of the pectoral fin; the posterior placed on the longitudinal series of scales just below the anterior section, between the vertical through the end of the pectoral fin and the middle portion of the caudal-fin base, with both segments having

one neuromast per scale". The author also noted that *A. haraldsiolii*, *A. janeiroensis*, *A. jurubatibensis*, *A. nudiventris*, *A. riograndensis*, and *A. santensis* share similar coloration patterns and belong to a group diagnosable by a lateral line divided into two sections in the same configuration. This group was designated the "*A. santensis* species group." *A. tupinambas* and *A. peruibensis* share the characteristics described above and are therefore considered part of the *A. santensis* species group.

In 1992, Huber proposed that *Rivulus santensis* might comprise multiple distinct species, describing two geographic patterns of caudal fin coloration in males: populations from northern Santos exhibited both dorsal and ventral black margins on the caudal fin, while those from southern Santos showed only a black ventral margin. However, these patterns were not confirmed in the present study, as they were observed across all examined species.

Our findings reveal major morphological distinctions within what Costa (2008) identified as the *A. santensis* species complex. The most significant are: a reduced number of pelvic-fin rays (5 *vs.* 6), a lower count of scales in the longitudinal series (33–34 *vs.* 35–36) compared to *A. santensis*, and a higher count of pectoral-fin rays (14 *vs.* 10–12) in *A. peruibensis*.

At the time of its previous redescription (Costa, 2008c), the species was defined as ranging from Paranaguá, Paraná (southernmost point), to Ubatuba, São Paulo (northernmost point). In the present work, supported by genetic and morphological analyses, we verified that *A. santensis* is confined to Baixada Santista and the municipality of São Paulo. Populations from the northern coast of São Paulo (Bertioga, São Sebastião, and Ubatuba) belong to the new species *A. tupinambas*, while populations from the southern coast (Peruíbe, Itanhaém, and Pedro de Toledo) belong to *A. peruibensis*, described herein.

Comparative material examined. Brazil. *Atlantirivulus simplicis*, LBP 25422, 6 males (22.5–32.2 mm SL), 1 female (27.7 mm SL), Rio de Janeiro, Paraty, 23°11'27.5"S 44°43'31.1"W, 11 Apr 2017, E. V. Ywamoto & D. Nielsen. *Atlantirivulus ribeirensis*, LBP 31527, 5 (10.3–16.1 mm SL), São Paulo, Juquiá, 24°21'03.6"S 47°41'00.6"W, 15 Mar 2017, E. V. Ywamoto.

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AUTHORS' CONTRIBUTION

Eric Venturini Ywamoto: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing-original draft, Writing-review and editing.

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Claudio Oliveira: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing-review and editing.

ETHICAL STATEMENT

Specimens were collected in agreement with Brazilian laws through SISBIO/MMA permit number 3245 and procedures for collection, maintenance and analyses followed the international guidelines for animal experiments through CEEAA IBB/UNESP protocol number 1058.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are included in the supplementary material of this article.

COMPETING INTERESTS

The authors declare no competing interests.

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