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Three new species of cloud fish of genus *Hypsolebias* (Cyprinodontiformes: Rivulidae) from rio São Francisco basins, Northeast Brazil

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Three new species are described from a temporary reservoir located in the upper drainage of the rio Paracatu, a tributary of the rio São Francisco basin, in the Minas Gerais State, Brazil. A high concentration of endemic species of annual fishes belonging to the *Hypsolebias trilineatus* species group has been recorded in a small area encompassing the plateaus associated with the upper drainage of the rio Paracatu. These species can be distinguished from other *Hypsolebias* species, except those belonging to the *H. trilineatus* complex, by the presence of a dark metallic ochre-yellow coloration on the anterior portion of the male flanks; absence of humeral marks, black blotches on the anterior portion of the male flanks; and light-red pectoral fins in males. Examination of *H. trilineatus* group specimens from the upper rio Paracatu revealed three new species that can be separated from their congeners based on the flank color pattern in males and differences in morphometric traits. Here, we describe three new species of the *H. trilineatus* species group and report the ongoing decline of habitat in their areas of occurrence in the northwestern region of Minas Gerais State. All three species were assessed as Critically Endangered (CR). A key to identification of the species of *Hypsolebias trilineatus* species group is provided.

Keywords: Annual fish, Identification key, New species, Temporary pool, Systematics.

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Três novas espécies são descritas a partir de um reservatório temporário localizado na bacia superior do rio Paracatu, um afluente da bacia do rio São Francisco, no estado de Minas Gerais, Brasil. Uma alta concentração de espécies endêmicas de peixes anuais, pertencentes ao grupo de espécies *Hypsolebias trilineatus*, foram registradas em uma pequena área que abrange os planaltos associados à bacia superior do rio Paracatu. Essas espécies podem ser distinguidas de outras espécies de *Hypsolebias*, exceto aquelas pertencentes ao complexo *H. trilineatus*, pela presença de uma coloração amarelo-ocre metálica escura na porção anterior dos flancos dos machos; ausência de marcas umerais; manchas pretas na porção anterior dos flancos dos machos; e nadadeiras peitorais vermelho-claras nos machos. O exame de espécimes do grupo *H. trilineatus* do alto rio Paracatu revelou três novas espécies que podem ser diferenciadas de suas congêneres com base no padrão de coloração dos flancos dos machos e em diferenças nas características morfométricas. Aqui, descrevemos três novas espécies do grupo *H. trilineatus* e relatamos o declínio contínuo do habitat em suas áreas de ocorrência na região noroeste do estado de Minas Gerais. Todas as três espécies foram classificadas como Criticamente Ameaçadas (CR). Uma chave para a identificação das espécies do grupo *Hypsolebias trilineatus* é fornecida.

Palavras-chave: Chave de identificação, Espécie nova, Peixe anual, Poça temporária, Sistemática.

INTRODUCTION

The *Hypsolebias* Costa, 2006 genus belongs to the Rivulidae fish family. Several species within this family exhibit an annual life cycle, inhabiting temporary pools that dry out completely during the dry season. These species deposit their eggs in the substrate, where the embryos enter a state of diapause to survive unfavorable conditions. At the end of the rainy season, the temporary pools dry up and all adult fish die, but their eggs survive and develop slowly during the dry season, waiting for the next rainy season. As soon as the rains start, the temporary pools refill, the embryos complete their development and the eggs hatch, initiating a new generation that will have its turn to grow, reproduce and keep the cycle going. In many Brazilian locations they are colloquially known as "cloud fish", a reference to the local curiosity regarding how fish can suddenly appear in temporary pools that were completely dry not long ago (Nielsen, 2008).

The rio Paracatu, approximately 300 km in length, is located within the Brazilian Cerrado biome. It crosses vegetation characterized by typical Cerrado (Savannah), Cerrado *sensu stricto*, and "Campo sujo" (open grassland with scattered shrubs). The sub-basin also contains ecosystems of riparian riverine forests and seasonally dry forests, as well as hydromorphic systems such as marginal lagoons, temporary pools, and seasonally hydromorphic fields (Vasconcelos, 2010). The rio Paracatu and its tributaries, located on the left bank of the upper rio São Francisco, represent a biodiversity hotspot for fish species of the genus *Hypsolebias*, totaling six known species: *H. alternatus* (Costa & Brasil, 1994), *H. auratus* (Costa & Nielsen, 2000), *H. fasciatus* (Costa & Brasil, 2006),

H. gibberatus (Costa & Brasil, 2006), *H. trilineatus* (Costa & Brasil, 1994), and *H. virgulatus* (Costa & Brasil, 2006).

The systematics of Rivulidae family has been frequently modified in the last years (Costa, 1995, 1998, 2006, 2011). The genus *Hypsolebias* was created in Costa, 2006. Initially, *Hypsolebias* was proposed as a subgenus of *Simpsonichthys*; however, after revisions based on morphological analyses (Costa, 2011), *Hypsolebias* was elevated to the category of genus. These subgenres were elevated to the category of genera in 2011. The *Hypsolebias genus* contains five monophyletic groups of species: *H. notatus* species group, *H. flammeus* species group, *H. magnificus* species group; *H. antenori* species group (Costa, 2007), and *H. fluminantis* species group (Costa *et al*., 2018).

Hypsolebias trilineatus, *H. auratus*, and *H. virgulatus* (Costa & Brasil, 2006) form a consistent subclade, herein referred to as the *Hypsolebias trilineatus* species group (the sixth recognized within the genus). This group is morphologically supported by prior phylogenetic hypotheses of the family Rivulidae (*e.g*., Costa, 2007, 2011). Herein, based on a comparative morphological approach following the standards of Costa (2007), this group is readily distinguished by a combination of unique morphological traits in males: a metallic dark yellow to ochre coloration on the anterior portion of the flanks, absence of humeral marks, the presence of black blotches on the anterior portion of flanks, and pale reddish pectoral fins. Females are characterized by the presence of dark gray vertical bars along the flanks (Costa, 2007). Notably, this is the only species complex within *Hypsolebias* that exhibits a golden yellow flank coloration in males. The three new species described in this study are assigned to this group based on these shared apomorphic color patterns and diagnostic morphological characters.

The apparent rarity of these species could be related to the lack of data from annual aquatic environments, in the upper rio Paracatu area and has been stagnant since 2006. However, in the last three years, this reality has changed and new field research has resulted in the discovery of these three new species. These surveys have also demonstrated drastic anthropogenic modifications of the typical Cerrado (Savannah) landscape, which has been replaced by agriculture without any study of the impact that these transformations have on annual aquatic environments.

An uncommonly high concentration of endemic species of cloud fishes has been recorded for a small area encompassing the highland plateaus associated with the upper sections of the rio Paracatu drainages, in the upper section of the rio São Francisco basin (Costa, Brasil, 1993, 1996; Costa, Nielsen, 2004; Costa, 2006). All species of the *H. trilineatus* species group currently known have them distribution in the basin of the rio Paracatu and its tributaries, ribeirão Taboca, rio Preto, and ribeirão Entre Ribeiros in an estimated area of 2,500 km^2. The three new species, described here is found in upper in drainage area of rio Paracatu, the tributary of left bank to the rio São Francisco.

MATERIAL AND METHODS

The biological data was measurements were taken point-to-point under a stereomicroscope with a digital caliper to the nearest 0.1 mm on the left side of the specimen following Costa (1995, 2007). Measurements are expressed as percents of standard length (SL), except subunits of the head, which are recorded as percents of head length (HL).

In the description, counts of vertebrae and pleural ribs were taken from cleared and stained (c&s) of eight males and seven females paratypes of each species, prepared according to Taylor, Van Dyke (1985). Frontal squamation followed Hoedeman (1958) and Costa (2006). For vertebral counts the caudal compounded centrum was counted as a single element. Osteological features included in the description are those considered phylogenetically informative in recent studies of the species formerly assigned to the genus *Simpsonichthys* (Costa 2003, 2007).

Institutional abbreviation is Museu de Diversidade Biológica, Universidade Estadual de Campinas (ZUEC). Comparisons with congeners were based primarily on the literature (Costa, 2007; Costa, Brasil, 1994, 2006; Costa, Nielsen, 2000; Nielsen *et al*., 2010).

RESULTS

Hypsolebias rosai, new species

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(Figs. 1–2; Tab. 1)

Holotype. ZUEC 18374, male, 28.2 mm SL, Brazil, Minas Gerais, Paracatu, temporary pool in drainage of the upper rio Paracatu, rio São Francisco basin, 17°04'05.48" S 46°26'39.98" W, 16 Mar 2023, T. Yoshinaga, F. O. de Lira & W. Ramalho.

Paratypes. ZUEC 18375, 23 males, 17.5–28.9 mm SL, 48 females, 14.5–19.7 mm SL, 5 c&s (3 males, 17.5–22.3 mm SL and 2 females, 16.8–18.2 mm SL), collected with holotype.

Diagnosis. *Hypsolebias rosai* differs from all other species of the *Hypsolebias* genus, except *H. trilineatus*, *H. virgulatus*, *H. auratus* color pattern in adults' males, sides of body light yellowish to golden with black blotches on anterior portion of flanks; pectoral fins pale red (*vs*. absent color pattern). Differs from *H. trilineatus*, *H. virgulatus*, *H. auratus* by the unique coloration pattern in the male flanks, which are golden with a reddish-brown background, with ten to eleven pale vertical black spots (*vs*. four to seven dark brown bars on posterior portion of flanks in *H. virgulatus*; flanks light yellowish-gray, golden on anterior third; three purplish-brown stripes, the median stripe continuous with a vertical row of three round black blotches on anterior portion of flanks in *H. trilineatus*; anterior third of side of body golden, with three or four rounded black blotches, posterior two thirds light purplish-brown, in *H. auratus*). In addition to coloration, *H. rosai* is distinguished from *H. trilineatus*, *H. virgulatus*, *H. auratus* by meristic characters, including a lower number of dorsal-fin rays (16–17 *vs*. 19–22 in congeners) and caudal-fin rays (22–23 *vs*. 24–28). Males also exhibit a distinct pelvic-fin length, with the tip of the pelvic-fin reaching the vertical through the base of the 1st anal-fin ray (*vs*. reaching the 2nd–3rd anal-fin ray in *H. virgulatus*, *H. auratus*, and the 4th-5th anal-fin ray in *H. trilineatus*). Females of *H. rosai* differ from *H. trilineatus*, *H. virgulatus*, *H. auratus* by the

position of the anal-fin origin, which is located posterior to the dorsal-fin base, on a vertical line through the base of the 4th to 5th dorsal-fin rays (*vs*. anal-fin origin anterior to the dorsal-fin, on vertical line through the base of 2nd to 3rd dorsal-fin rays in *H. virgulatus*, *H. trilineatus,* and through the base of the 1st ray in *H. auratus*).

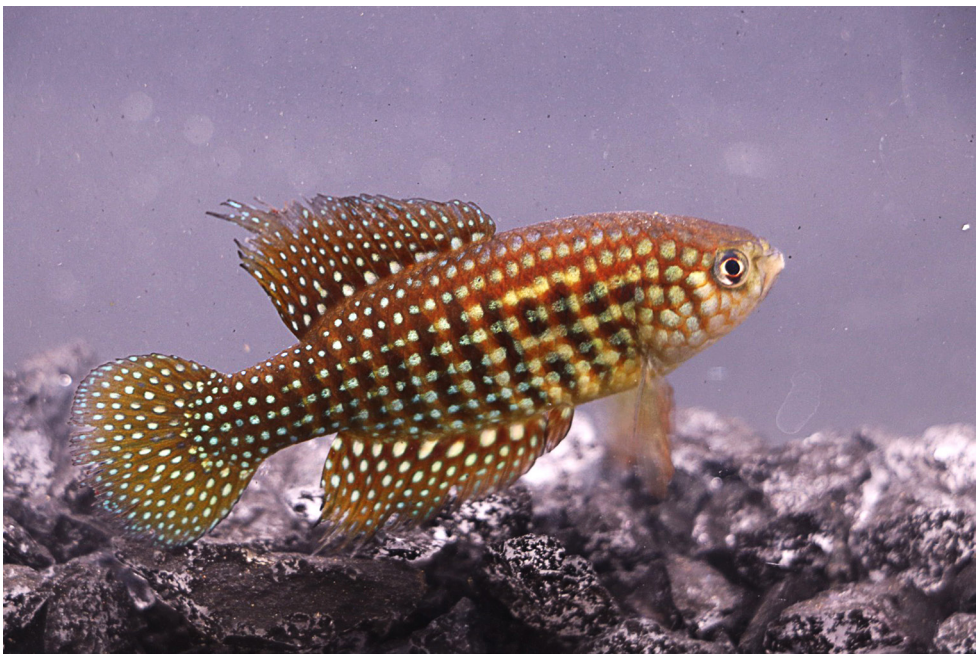


FIGURE 1 | *Hypsolebias rosai*, ZUEC 18374, male, holotype, 28.2 mm SL: Brazil, Minas Gerais, Paracatu. Photo by Thomas Yoshinaga.



FIGURE 2 | *Hypsolebias rosai*, ZUEC 18375, female, paratype, 18.6 mm SL: Brazil, Minas Gerais, Paracatu. Photo by Thomas Yoshinaga.

Description. Morphometric data is presented in Tab. 1. Largest male examined 28.9 mm SL; largest female 19.7 mm SL. Dorsal profile convex from snout to the end of the dorsal-fin base, becoming approximately straight along the caudal peduncle. Body deep is compressed, body depth at level of pelvic-fin base. Snout short. Urogenital papilla cylindrical and short in males, pocket-shaped in females.

Tip of dorsal-fin pointed in males, with 2–3 filaments, and slightly pointed in females. Anal-fin pointed in males, with 2–3 filaments, and while rounded in females. Dorsal-fin rays unbranched in both sexes. Tip of filamentous rays on dorsal and anal fins in males reaching vertical through half of caudal fin.

Caudal-fin rounded. Pectoral-fins elliptical. Posterior margin of each pectoral-fin reaching vertical between base of 3rd anal-fin ray in males and reaching between the pelvic-fin base and the urogenital papilla in females. Tip of pelvic-fin reaching base of the 1st anal-fin ray in both sexes. Dorsal-fin origin positioned posterior to the anal-fin origin in males, and aligned vertically with the base of the 1st–2nd anal-fin rays, in females. Dorsal-fin origin located between the neural spines of the 9th–10th vertebrae in males, and the neural spines of the 12th–13th vertebrae in females. Anal-fin origin aligns with the pleural ribs of the 11th vertebrae in males, and 12th in females. Dorsal-fin rays 16–17 in males, 13–14 in females; anal-fin rays 17–18 in males, 15–16 in females; caudal-fin rays 22–23; pectoral-fin rays 13–14; pelvic-fin rays 6.

Frontal squamation is A-patterned; with E-scales overlapping medially. Longitudinal scales series 26–27 scales; transverse series 9–10; and 12 scales around caudal peduncle. Contact organs absent of flank and pectoral-fin in males.

Cephalic neuromasts supraorbital 12–14, parietal two, anterior rostral one, posterior rostral one, infraorbital 2+18, preorbital three, otic two, post-otic one, supratemporal one, median opercular one, ventral opercular two, preopercular 14–15, mandibular 11, lateral mandibular five, and paramandibular one. Vomerine teeth absent. Two neuromasts present at the caudal-fin base. Vertebral 24–25.

TABLE 1 | Morphometric data for the holotype and paratypes of *Hypsolebias rosai*.

	Holotype	Paratypes	Paratypes
	Male	**Males (n = 23)**	**Females (n = 48)**
Standard length (mm)	28.2	17.5–28.9	14.5–19.7
Percents of standard length			
Body depth	32.2	29.6–35.8	32.1–35.7
Caudal peduncle depth	15.9	15.5–18.1	13.6–15.6
Pre-dorsal length	53.2	50.0–62.3	60.5–68.4
Pre-pelvic length	45.4	44.2–50.7	48.2–53.1
Length of dorsal-fin base	34.7	30.5–35.2	18.0–22.1
Length of anal-fin base	31.9	29.6–33.0	18.7–20.5
Caudal-fin length	28.6	27.6–31.8	26.7–33.5
Pectoral-fin length	21.3	20.4–28.8	18.5–26.1
Pelvic-fin length	8.4	7.7–9.7	8.1–9.5
Head length	32.4	30.5–33.1	34.7–37.1
Percents of head length			
Head depth	100.0	98.3–100.4	86.8–92.2
Head width	66.8	65.3–70.5	64.3–69.2
Snout length	10.8	10.8–13.8	14.5–17.6
Lower jaw length	22.7	21.6–24.1	26.4–29.0
Eye diameter	32.5	30.5–37.9	31.2–39.3

Coloration in life. The flanks are golden with a reddish-brown background, with 10–11 pale vertical black spots (Figs. 1–2). There are 6–9 horizontal rows formed by irregular golden dots, with different shapes and sizes, sometimes interconnecting along the entire flank, from the back to the portion above the belly. These spots are interspersed with reddish brown bands, and stronger tones in the region of the back and reddish-brown back, white to golden venter. Side of the head brown, opercular region golden with reddish-brown scale margins on the posterodorsal portion of the head. Iris yellowish-brown, featuring a distinct vertical black bar across the center of the eye. Dorsal-fin dark reddish-brown, with golden spots; caudal-fin orange brown with golden spots. Anal-fin reddish-brown at the base and orange brown in distal area with rounded golden spots. Spots at the base of the dorsal and anal fins are larger than those in the distal portions. Pelvic fins reddish-brown with iridescent golden-yellow spots. Pectoral fins are hyaline, with subtle brown pigmentation along the distal margins.

Females. The sides of the body are yellowish-gray, with irregular dark gray black spots; those positioned above the anal-fin base are often elongated, forming short vertical bars. Back and opercular region are grayish-brown, with dark brown scale margins that clearly outline the scales. Venter light gray. Iris is light yellow, featuring a distinct vertical black bar across the center of the eye. All fins are hyaline, with small black spots in the basal region (Fig. 2).

Geographical distribution. It is known only from the type locality, a temporary pool in drainage of the upper rio Paracatu, tributary right bank of the rio São Francisco basin in Santa Rosa region, Paracatu municipality, Minas Gerais State, Brazil (Fig. 3).

Ecological notes. Temporary pool with an area about 53.000 m^2 and 0.8 m maximum depth. The water had a variety of colors, in the beginning of the rainy season, the pool was white mud-colored, in the end of the rainy season, the water became translucent. Measured physicochemical parameters were pH 6.77, water temperature 30.3 °C, and conductivity 0.03 µS/cm. The region is characterized as Cerrado (Brazilian savannah) characterized by small trees with twisted trunks and branches, thick bark, and deep roots. It exhibits great structural variation, ranging from open formations (grasslands) to dense forests. In the pool present aquatic macrophytes present in deeper areas and abundant native grass along the floodplain.

Conservation status. *Hypsolebias rosai* is a new species endemic to the upper rio Paracatu drainage. They were registered in areas experiencing significant habitat degradation due to intensive agricultural activities. Part of the temporary pool area has already been filled for the expansion of soybean crops. The primary threats include drainage of flooded areas for agricultural, leading to a substantial reduction in both habitat quality and extent. Given these threats, the species' high level of endemism, and field observations indicating ongoing habitat loss, we suggest classifying the species as Critically Endangered (CR), B1ab(ii,iii) according to the IUCN Red List Categories and Criteria (IUCN, 2012, 2024), due to an extent of occurrence (EOO) estimated to be less than 100 km^2 (B1), the species being known to exist at only a single location (a), and a continuing decline observed or projected in the area of occupancy (b(ii)) and quality of habitat (b(iii)).

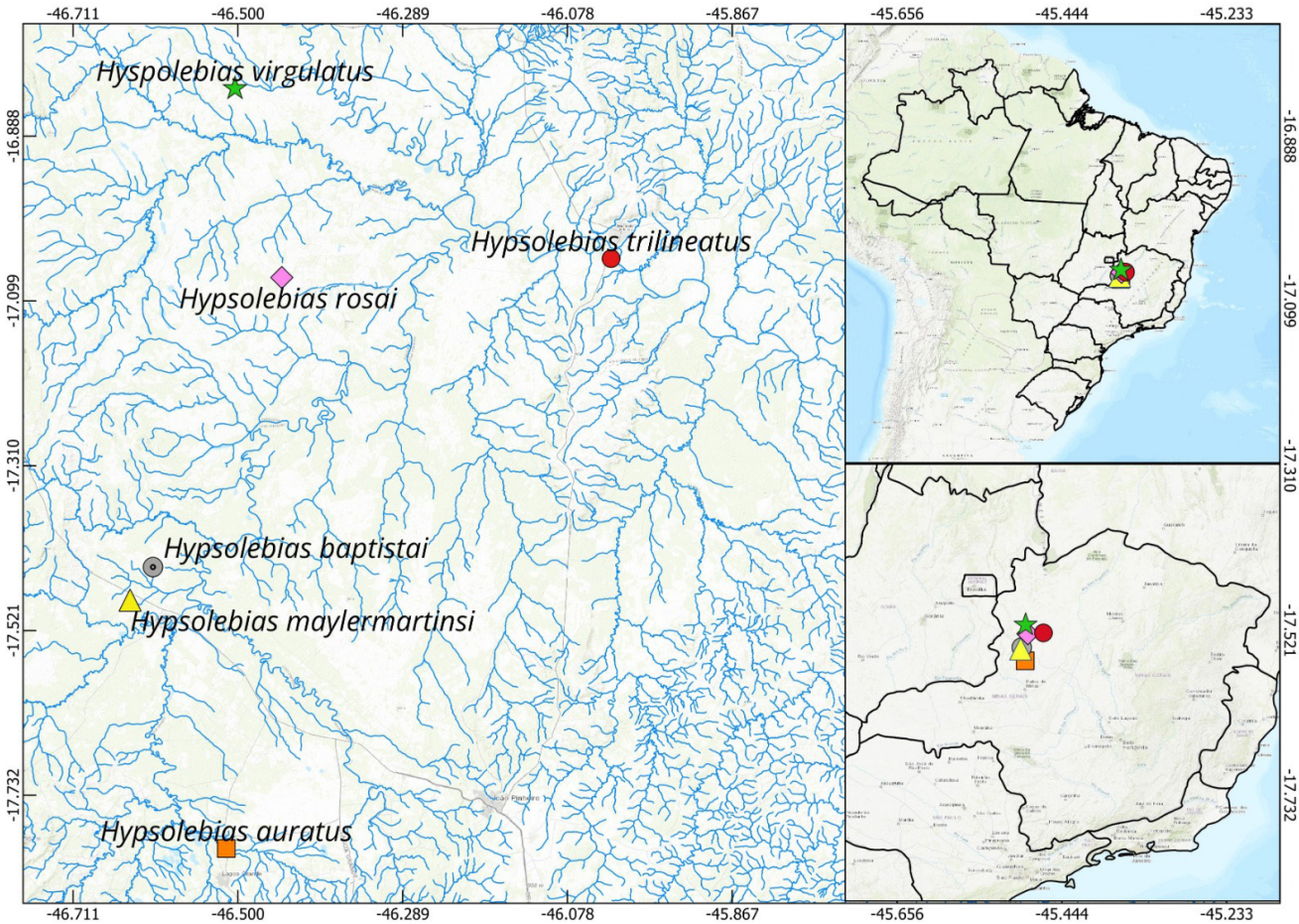


FIGURE 3 | Left, geographic distribution of the *Hypsolebias trilineatus* species group in the upper rio Paracatu drainage, São Francisco River basin, Brazil. Upper right, location of the study area in Brazil. Lower right, enlarged view of the northwestern region of Minas Gerais State showing the distribution of the species.

Etymology. The specific epithet honors of João Guimarães Rosa was a Brazilian poet, diplomat, novelist, short story writer and physician, considered by many to be the greatest Brazilian writer of the 20th century and one of the greatest of all time. His most notable work was "Grande Sertão Veredas", which is set in the region where the new species, occurs. A noun in a genitive case.

Hypsolebias baptistai, new species

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(Figs. 4–5; Tab. 2)

Holotype. ZUEC 18376, male, 46.7 mm SL, Brazil, Minas Gerais, Paracatu, rio Paracatu drainage, rio São Francisco basin, 17°26'28.3" S 46°36'33.8" W, 16 Mar 2023, F. O. de Lira, T. Yoshinaga & L. E. K. Lanés.



FIGURE 4 | *Hypsolebias baptistai*, ZUEC 18376, male, holotype, 46.7 mm SL: Brazil, Minas Gerais, Paracatu. Photo by Dalton T. B. Nielsen.



FIGURE 5 | *Hypsolebias baptistai*, ZUEC 18377, female, holotype, 38.7 mm SL: Brazil, Minas Gerais, Paracatu. Photo by Dalton T. B. Nielsen.

Paratypes. ZUEC 18377, 30 males, 20.2–38.7 mm SL, 26 females, 17.0–36.0 mm SL; 5 c&s (2 males, 35.1–36.6 mm SL and 3 females, 27.9–29.5 mm SL), collected with holotype.

Diagnosis. *Hypsolebias baptistai* differs from all other species of the *Hypsolebias* genus, except *H. trilineatus, H. virgulatus*, *H. auratus* color pattern in adults'males, sides of body light yellowish to golden with black blotches on anterior portion of flanks; pectoral fins pale red (*vs*. absent color pattern). Differs from *H. trilineatus*, *H. virgulatus*, *H. auratus* by the unique coloration pattern in the male flanks with the lateral surface golden, displaying three large and irregular black blotches of flanks between the opercle and the origin of the dorsal-fin (*vs*. 4–7 dark brown bars on posterior portion of flanks in *H. virgulatus*; flanks light yellowish-gray, golden on anterior 3rd; 3 purplish-brown stripes, the median stripe continuous with a vertical row of 3 round black blotches on anterior portion of flanks in *H. trilineatus*; anterior 3rd of side of body golden, with 3–4 rounded black blotches, posterior two thirds light purplish-brown, in *H. auratus*). In addition to coloration, *H. baptistai* is distinguished from *H. trilineatus*, *H. virgulatus*, *H. auratus* by anal-fin origin is located posterior to dorsal-fin origin, on a vertical through the base of 5th–6th dorsal-fin rays (*vs.* 2nd–3rd in *H. virgulatus*, *H. trilineatus*, *H. auratus*), greater lower jaw length 26.4–29.0% HL (*vs*. 12.9–23.2%), in males.

Description. Morphometric data are presented in Tab. 2. Largest male examined 46.7 mm SL, largest female 36.0 mm SL. Dorsal profile is convex from snout to the end of the dorsal-fin base, becoming approximately straight along the caudal peduncle. Body deep compressed, body depth at level of the pelvic-fin base. Snout short. Urogenital papilla cylindrical and short in males, and pocket-shaped in females.

Tip of the dorsal-fin pointed in males, with 2–3 filaments, and slightly pointed in females. Anal-fin is rounded in males, with 2–3 filaments, and while rounded in females. Dorsal-fin rays unbranched in both sexes. Tip filaments rays on dorsal fin reach vertically through the end of the caudal-fin length, caudal fin short filaments.

Caudal-fin rounded. Pectoral fins are elliptical. Posterior margin of the pectoral-fin reaches the vertical through the base of the 3rd anal-fin ray, in males and reaches between the pelvic-fin base and the urogenital papilla, in females. Tip of the pelvic-fin reaches base of the 3rd anal-fin ray in males, and base of the 1st anal-fin ray in females.

Dorsal-fin origin positioned posterior to the anal-fin origin in males, and aligned vertically with base of the 1st to 2nd anal-fin rays in females. Dorsal-fin origin located between the neural spines of the 7th–8th vertebrae in males, and the neural spines of the 10th–11th vertebrae in females. Anal-fin origin, which is located posterior to the dorsal-fin base, on a vertical line through base of the 5th–6th dorsal-fin rays in males; and on a vertical through base 4th–5th dorsal-fin rays in females. Anal-fin origin aligns with the pleural ribs of the 11th vertebrae in males, and 10th in females. Dorsal-fin rays 21–22 in males, 15–16 in females; anal-fin rays 20–21 in males, 17–18 in females; caudal-fin rays 24–26; pectoral-fin rays 13–14; pelvic-fin rays 6.

Frontal squamation is A-patterned; with E-scales overlapping medially. Longitudinal series contains 26–27 scales; transverse series eight to nine; and 12 scales around caudal peduncle. Contact organs absent of flank and pectoral-fin in males.

TABLE 2 | Morphometric data for the holotype and paratypes of *Hypsolebias baptistai*.

	Holotype	Paratypes	Paratypes
	Male	Males (n = 30)	Females (n = 26)
Standard length (mm)	46.7	20.2–38.7	17.0–36.0
Percents of standard length			
Body depth	36.8	31.7–37.2	32.2–37.2
Caudal peduncle depth	17.1	15.1–16.7	12.8–17.5
Pre-dorsal length	45.8	48.2–53.1	62.0–67.7
Pre-pelvic length	46.2	37.0–48.6	47.0–54.7
Length of dorsal-fin base	35.1	28.3–35.5	20.8–26.9
Length of anal-fin base	31.0	28.3–32.2	19.4–21.3
Caudal-fin length	28.2	30.0–34.4	30.5–34.4
Pectoral-fin length	21.8	24.0–28.6	21.3–25.8
Pelvic-fin length	9.8	8.1–10.6	6.9–11.0
Head length	31.7	28.6–32.7	29.4–33.5
Percents of head length			
Head depth	91.9	89.1–95.7	84.0–91.4
Head width	64.6	62.1–86.6	65.1–70.2
Snout length	17.6	15.7–20.0	12.3–18.8
Lower jaw length	26.3	24.2–29.0	17.1–27.0
Eye diameter	33.7	30.1–36.9	29.7–34.2

Cephalic neuromasts counts are: supraorbital 12–13, parietal two, anterior rostral one, posterior rostral one, infraorbital 4+19–20, preorbital three, otic one, post-otic two, supratemporal one, median opercular one, ventral opercular one, preopercular 12–14, mandibular 9–10, lateral mandibular five, and paramandibular one. Vomerine teeth are absent. Two neuromasts are present at the caudal-fin base. Vertebrae count is 27–28.

Coloration in life. Males. Flanks golden ground-color, featuring 3 large black blotches anteriorly between opercle and dorsal-fin origin. Lateral pigmentation consists of 6 horizontal lines of golden-yellow scales transitioning to bright greenish-blue posterior to dorsal-fin origin, interspersed with 7 narrow, reddish-brown and yellow stripes; dorsal region with darker undertones. Faint vertical bars 7–8 present from 1st anal-fin rays to caudal peduncle. Greenish-blue dots form 6–7 horizontal series along entire flank, decreasing to 4–5 series at caudal peduncle. Dorsum light brown, venter light yellowish. Side of head golden-yellow laterally; opercular region intensely golden with geometric patterns delimited by brown lines. Postero-dorsal scale margins orange-brown. Iris light yellow, vertical reddish-brown bar through center. Dorsal and anal fins pale reddish-brown, bearing rounded greenish-blue spots that decrease in size distally. Caudal fin light reddish-brown, small greenish-blue spots. Pelvic fins light yellow basally, becoming reddish-brown distally. Pectoral fins hyaline with brown margins (Fig. 4).

Females. Flanks light yellowish-gray, covered with irregular dark gray to black spots; spots dorsal to anal-fin base frequently elongated, forming short vertical bars. Dorsum grayish-brown; venter light yellow. Opercular region iridescent greenish. Iris light yellow, with a distinct vertical gray bar through center. Unpaired fins hyaline, bearing small black spots basally. Pelvic and pectorais fins hyalines (Fig. 5).

Geographical distribution. It is known only from the type locality, a temporary pool in drainage of the upper rio Paracatu, tributary left bank rio Paracatu, rio São Francisco basin in Santo Antônio dos Olhos D'água region, Paracatu municipality, Minas Gerais State, Brazil (Fig. 3).

Ecological notes. The new species was collected in a temporary pool with an area about 77.000 m² and 1.0 m maximum depth. The water had a variety of colors, in the beginning of the rainy season, the pool was white mud-colored, in the end of the rainy season, the water became translucent. Measured physicochemical parameters were pH 6.83, water temperature 29.2 °C, and conductivity 0.01 µS/cm. The region is characterized as Cerrado (Brazilian savannah), characterized by small trees with twisted trunks and branches, thick bark, and deep roots. It exhibits great structural variation, ranging from open formations (grasslands) to dense forests with some aquatic macrophytes present in deeper areas and abundant native grass along the floodplain.

Conservation status. *Hypsolebias baptistai* is a new species endemic to the upper rio Paracatu drainage. They were registered in areas experiencing significant habitat degradation due to intensive agricultural activities. Part of the temporary pool area has already been filled for the expansion of soybean crops. The primary threats include drainage of flooded areas for agricultural, leading to a substantial reduction in both habitat quality and extent. Given these threats, the species' high level of endemism, and field observations indicating ongoing habitat loss, we suggest classifying the species as Critically Endangered (CR), B1ab(ii,iii) according to the IUCN Red List Categories and Criteria (IUCN, 2012, 2024), due to an extent of occurrence (EOO) estimated to be less than 100 km² (B1), the species being known to exist at only a single location (a), and a continuing decline observed or projected in the area of occupancy (b(ii)) and quality of habitat (b(iii)).

Etymology. The specific epithet is in honor of environmentalist and fish veterinarian Arsênio Caldeira Baptista Junior. A noun in a genitive case.

Hypsolebias maylermartinsi, new species

urn:lsid:zoobank.org:act:E8951019-5FFA-4B9F-B0A4-EA68CE761F7C

(Figs. 6–7; Tab. 3)

Holotype. ZUEC 18372, male, 41.1 mm SL, Brazil, Minas Gerais, Paracatu, rio Paracatu drainage, rio São Francisco basin, 17°28'51.70" S 46°38'15.09" W, 17 Mar 2023, D. T. B. Nielsen, T. Yoshinaga, F. O. de Lira, L. E. K. Lanés & W. Ramalho.

Paratypes. ZUEC 18373, 13 males, 20.7–28.6 mm SL, 18 females, 18.3–27.4 mm SL, 5 c&s (3 males, 25.8–27.5 mm SL and 2 females, 18.2–26.4 mm SL, collected with holotype.

Diagnosis. *Hypsolebias maylermartinsi* differs from all other species of the *Hypsolebias* genus, except *H. trilineatus*, *H. virgulatus*, *H. auratus* color pattern in adults' males, sides of body light yellowish to golden with black blotches on anterior portion of flanks; pectoral fins pale red (*vs*. absent color patter). Differs from *H. trilineatus*, *H. virgulatus*, *H. auratus* by the unique coloration pattern if male flanks, which are golden with 6–7 yellow lines in the region between the head and the 1st dorsal-fin ray, followed posteriorly formed by bright greenish-blue spots interspersed with five brown lines, the central one being black, extending from the head to the beginning of the dorsal-fin (*vs.* four to seven dark brown bars on posterior portion of flanks; vertical rows of light green dots along whole flank in *H. virgulatus*; flanks light yellowish-gray, golden on anterior third, with three purplish-brown stripes, the median stripe continuous with a vertical row of three round black blotches on anterior portion of the flanks; six horizontal rows of white dots on posterior two thirds of flanks, in *H. trilineatus*; anterior third of side of body golden, with three or four rounded black blotches, posterior two thirds light purplish-brown, with dark purplish-brown bars and six to eight horizontal rows of light greenish-blue dots, in *H. auratus*). It also presents a lower number of longitudinal scale series 23–24 (*vs*. 25–28) and a lower number of transverse scale series 6–7 (*vs.* 8–10). Additionally, *H. maylermartinsi* can be distinguished from *H. virgulatus*, *H. trilineatus* and *H. auratus* by the dorsal-fin origin being located between the neural spines of the 10th–11th vertebrae (*vs*. 7th–9th), anal-fin origin between the pleural ribs of vertebrae 10th–11th (*vs*. 7th–9th); and by the absence of contact organs (*vs*. presence of small papillate contact organs on the upper surface of the dorsal most ray of pectoral fins, in *H. virgulatus* and *H. trilineatus*, contact organ on each scale of the anteroventral portion of flank, in *H. auratus*), in males.



FIGURE 6 | *Hypsolebias maylermartinsi*, ZUEC 18372, male, holotype, 41.1mm SL: Brazil, Minas Gerais, Paracatu. Photo by Thomas Yoshinaga.

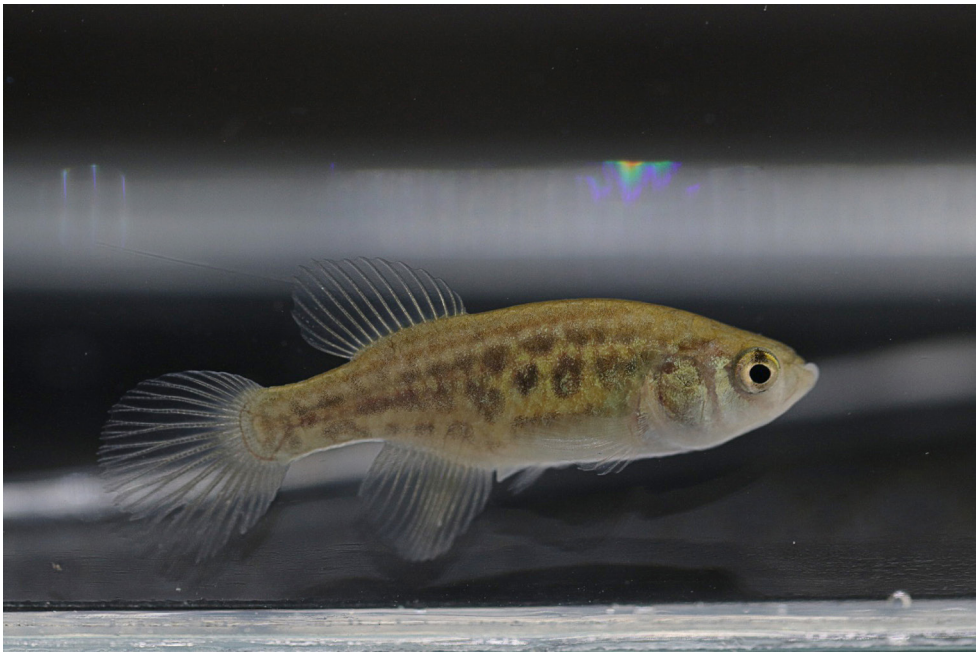


FIGURE 7 | *Hypsolebias maylermartinsi*, ZUEC 18373, female, holotype, 27.1 mm SL: Brazil, Minas Gerais, Paracatu. Photo by Thomas Yoshinaga.

Description. Morphometric data are presented in Tab. 3. Largest male examined 28.6 mm SL; largest female 27.4 mm SL. Dorsal profile is convex from snout to the end of the dorsal-fin base; becoming approximately straight along the caudal peduncle. Body deep is compressed, body depth at level of the pelvic-fin base. Snout short. Urogenital papilla is cylindrical and short in males, pocket-shaped in females.

Tip of dorsal-fin is pointed in males, with 2–3 filaments, and slightly pointed in females. Anal-fin is pointed in males, with 2–3 filaments, and while rounded in females. Dorsal-fin rays are unbranched in both sexes. Filaments rays of dorsal and anal fins in males, tip of filaments reaching vertically through half the length of the caudal peduncle.

Caudal-fin is rounded, and the pectoral fins are elliptical. Posterior margin of the pectoral-fin reaching vertical through base of the 5th anal-fin ray in males and reaching between pelvic-fin base and urogenital papilla in females. Tip of pelvic-fin reaching base of the 3rd anal-fin ray in males, and base of the 1st anal-fin ray in females.

Dorsal-fin origin at the vertical through the base of the 2th anal-fin ray in females. Dorsal-fin origin located between neural spines of the 10th–11th vertebrae in males, and between neural spines of the 11th–12th vertebrae in females. Anal-fin origin, which is located posterior to dorsal-fin base, on a vertical line through base of the 2th–3th dorsal-fin ray in males. Anal-fin origin is between the pleural ribs of vertebrae 10th–11th in males, and between the pleural ribs of vertebrae 12th–13th in females. Dorsal-fin rays 19–20 in males, 14–15 in females; anal-fin rays 19–20 in males, 16–17 in females; caudal-fin rays 24–25; pectoral-fin rays 13–14; pelvic-fin rays 6.

Frontal squamation is A-patterned; with E-scales overlapping medially. Longitudinal series contains 23–24 scales; transverse series six to seven; and 12 scales around caudal peduncle. Contacts organs absent of flank and pectoral-fin in males.

TABLE 3 | Morphometric data for the holotype and paratypes of *Hypsolebias maylermartinsi*.

	Holotype	Paratypes	Paratypes
	Male	Males (n = 11)	Females (n = 17)
Standard length (mm)	41.1	20.7–28.6	18.3–27.4
Percents of standard length			
Body depth	30.7	28.3–32.0	29.7–32.7
Caudal peduncle depth	15.3	14.3–15.9	13.6–14.9
Pre-dorsal length	49.5	49.4–52.3	60.9–66.3
Pre-pelvic length	42.7	42.7–48.8	50.7–54.5
Length of dorsal-fin base	35.2	31.4–39.3	20.9–24.9
Length of anal-fin base	33.4	28.6–34.4	19.7–22.4
Caudal-fin length	31.8	27.1–31.7	30.7–31.3
Pectoral-fin length	23.7	22.7–26.1	23.3–25.4
Pelvic-fin length	7.7	6.2–8.7	7.2–11.4
Head length	30.8	30.3–31.6	31.4–34.6
Percents of head length			
Head depth	86.3	85.2–92.8	79.4–92.4
Head width	68.3	66.7–72.3	63.5–68.4
Snout length	15.5	15.5–18.7	12.8–14.9
Lower jaw length	24.8	23.4–28.0	17.7–23.2
Eye diameter	32.4	30.5–32.5	28.6–30.7

Cephalic neuromasts counts are: supraorbital 11, parietal two, anterior rostral one, posterior rostral one, infraorbital 4+21–22, preorbital three, otic one, post-otic two, supratemporal one, median opercular one, ventral opercular one, preopercular 12–13, mandibular 9–10, lateral mandibular five, paramandibular one. Vomerine teeth are absent. Two neuromasts are present at the caudal-fin base. Vertebrae count is 25–26.

Coloration in life. Males. Flanks golden ground-color, 6–7 yellow lines anteriorly between head and first dorsal-fin ray; posterior region featuring bright greenish-blue spots interspersed with five brown lines extending from opercular opening to dorsal-fin origin, the central line black. Dorsum light brown; venter yellowish-white. Head golden laterally; opercular region intensely golden. Postero-dorsal scale margins orange-brown. Iris light yellow, vertical reddish-brown bar through center. Dorsal and anal fins pale reddish-brown, bearing rounded greenish-blue spots that are larger basally and merge into small, elongated spots distally. Caudal fin light reddish-brown, with small greenish-blue spots. Pelvic fins reddish-brown with a light-yellow base. Pectoral fins pale red. Pelvic and Pectoral fins without spots (Fig. 6).

Females. Flanks light yellowish-gray, 1–3 rounded gray spots on anterocentral region, surrounded by irregular spots occasionally extending to caudal-fin base or forming vertical lines. Dorsum grayish-brown; venter light yellow. Opercular region iridescent greenish. Iris light yellow, vertical black bar through center. Fins hyaline (Fig. 7).

Geographical distribution. It is known only from the type locality, a temporary pool in drainage of the upper rio Paracatu, rio São Francisco basin in Frederico region, Paracatu municipality, Minas Gerais State, Brazil (Fig. 3).

Ecological notes. The new species was collected in a temporary pool with an area about 1.520 m^2 and 1.0 maximum depth during the rainy season. The water was turbid at the beginning of the rainy season and translucent at the end. Measured physicochemical parameters were pH 6.02, water temperature 31.8 °C, and conductivity 0.01 µS/cm. The region is characterized as Cerrado (Brazilian savannah), with some aquatic macrophytes present in deeper areas and abundant native grass along the floodplain.

Conservation status. *Hypsolebias maylermartinsi* is a new species endemic to the upper rio Paracatu drainage They were registered in areas experiencing significant habitat degradation due to intensive agricultural activities. The main threats include erosion and excessive sedimentation in the drainage areas. The pool was half excavated to retain water during the dry season and is used for livestock watering. Consequently, the species is also exposed to the risk of transport accidents involving toxic cargo. Given these threats, the species' high level of endemism, and field observations indicating ongoing habitat loss, we suggest classifying the species as Critically Endangered (CR), B1ab(ii,iii) according to the IUCN Red List Categories and Criteria (IUCN, 2012, 2024), due to an extent of occurrence (EOO) estimated to be less than 100 km^2 (B1), the species being known to exist at only a single location (a), and a continuing decline observed or projected in the area of occupancy (b(ii)) and quality of habitat (b(iii)).

Etymology. The specific epithet is in honor of environmentalist and biologist Mayler Martins. A noun in a genitive case.

Key to species of the *Hypsolebias trilineatus* species group (males)

1a. Anterior portion of the flanks with 7–13 black blotches; pectoral fins hyaline; pelvic fin yellowish brown..... *H. virgulatus*

1b. Anterior portion of the flanks with 3–4 black blotches rounded or vertical (almost a bar); pectoral fin ventral edges pale orange or brown; pelvic fin reddish brown..... 2

2a. Pelvic-fin with five rays; posterior portion of flank with three purplish-brown stripes; no dark brown blotch on the anteroventral portion of the flank ..... *H. trilineatus*

2b. Pelvic-fin with six rays; posterior portion of flank light purplish brown, with dark purplish brown bars and 6–8 horizontal rows of light greenish blue dots or reddish-brown background with pale vertical black spots and irregular golden dots or light brown with 4–5 lines formed by greenish-blue dots or golden with lines formed by bright greenish-blue spots interspersed with five brown lines; anteroventral portion of the flank golden with dark brown blotch 3

3a. Posterior portion of flank with vertical purplish-brown bars; dark brown blotch on the anteroventral portion of the flank..... *H. auratus*

3b. Posterior portion of flank with horizontal reddish bars or dark reddish with golden dots; white anteroventral portion of the flank or white scales with red brown outline 4

4a. Dorsal-fin with 16–17 rays; 10–11 pale vertical black spots on the posterior portion of the flank..... *H. rosai*

4b. Dorsal-fin with 19–22 rays, posterior portion of flank with alternating horizontal lines of bright blue and red brown 5

5a. Anal-fin origin posterior to dorsal-fin origin; 7–8 pale brown vertical bars, almost imperceptible; anal-fin origin aligned with 5th–6th dorsal-fin ray ..... *H. baptistai*

5b. Longitudinal series with 23–24 scales; 6–7 transverse series of scales; central line black on anterior portion of the flank ..... *H. maylermartinsi*

DISCUSSION

In 2007, Costa proposed the creation of four monophyletic species group within the subgenus *Hypsolebias* (*Simpsonichthys* genus): the *H. notatus* species group, the *H. flammeus* species group. Additionally, in 2018 (when *Hypsolebias* was already being treated as a genus), was proposed another, *H. fluminantis* species group (Costa, 2007; Costa *et al*., 2018).

Hypsolebias trilineatus, *H. auratus*, and *H. virgulatus* form a consistent subclade, herein referred to as the *H. trilineatus* species group (the sixth recognized within the genus). This group is morphologically supported by prior phylogenetic hypotheses of Rivulidae (*e.g*., Costa, 2007, 2011). Herein, based on a comparative morphological approach following the standards of Costa (2007), this group is readily distinguished by a combination of unique morphological traits in males: a metallic dark yellow to ochre coloration on the anterior portion of the flanks, absence of humeral marks, the presence of black blotches on the anterior portion of flanks, and pale reddish pectoral fins. Females are characterized by the presence of dark gray vertical bars along the flanks (Costa, 2007). Notably, this is the only species complex within *Hypsolebias* that exhibits a golden yellow flank coloration in males. The three new species described in this study are assigned to this group based on these shared apomorphic color patterns and diagnostic morphological characters.

All three known species have their distribution restricted to just one population with a very narrow distribution range (Costa, 2007), all relatively close to the locations of the other three species already described in the *H. trilineatus* species group. The apparent rarity of these species group could be related to the lack of data from annual aquatic environments and has been stagnant since 2006.

However, in the last three years, this reality has changed and new field research has resulted in the discovery of these three new species. These surveys have also demonstrated drastic anthropogenic modifications of the typical Cerrado (Savannah) landscape, which has been replaced by agriculture without any study of the impact that these transformations have on annual aquatic environments (CITA).

Hypsolebias rosai is easily distinguished from its congeners by a unique male color pattern, with a strong golden and brown background and numerous iridescent yellow spots; *H. baptistai* is easily distinguished from its congeners by a golden coloration, with three large irregular black spots in the central region of the body between the operculum and the beginning of the dorsal-fin, and 7–8 pale brown vertical bars, almost imperceptible, starting at the first rays of the anal-fin and extending to the caudal peduncle; *H. maylermartinsi* is easily identified by the presence of 6–7 yellow lines in

the region between the head and the first dorsal-fin ray, followed by bright blue-green spots interspersed with five brown lines, the central line being black, extending from the head to the beginning of the dorsal-fin.

The color pattern of males is a key characteristic for diagnosing species within the Aplocheiloidei. Females recognize males by their color patterns to assess potential mates (Costa, 1995; Wildekamp, 2004; Britzke *et al*., 2016). There are very clear differences among the three new species. *Hypsolebias rosai* has a predominantly dark brown flank with numerous multicolored spots and a vertical series of black bars, giving it a color pattern more similar to *H. virgulatus* (Fig. 8), while the predominance of vertical lines in *H. baptistai* and *H. maylermartinsi* more closely resembles the color patterns of *H. trilineatus* (Fig. 9) and *H. auratus* (Fig. 10).

The rio Paracatu basin is located in low-lying areas of the rio São Francisco basin, at elevations between 400 and 600 m, and is characterized by the presence of both annual and non-annual pools and paths. The origin of the horizontal evolution of this depression began during the Cretaceous period (140–65 Mya), when drainage incision dissected the existing formations, eventually reaching the rocks of the Bambuí Group (IGAM, 2006). Sedimentary aquifer areas formed on these flat surfaces during the Tertiary and Quaternary periods, where regions with high densities of temporarily flooded areas are recorded (Vasconcelos *et al*., 2012). Some of these temporary floods are believed to have originated from aquifer outcrops, rainfall, or the overflow of rivers and streams over shallow clay depressions, with significant water loss due to evaporation (CETEC-MG, 1981; Ruralminas, 1996).



FIGURE 8 | *Hypsolebias virgulatus*, male, 37.1 mm SL: Brazil, Minas Gerais, Unaí. Photo by Maurice Chauche.



FIGURE 9 | *Hypsolebias trilineatus*, male, 35.7 mm SL: Brazil, Minas Gerais, Brasilândia de Minas. Photo by Maurice Chauche.



FIGURE 10 | *Hypsolebias auratus*, male, 36.6 mm SL: Brazil, Minas Gerais, Lagoa Grande. Photo by Didier Pillet.

The historical formation of these low-lying surfaces in the rio Paracatu basin favored the establishment of persistent annual aquatic environments, characterized by modern aquifer outcrops and seasonal floodings over clay depressions (CETEC-MG, 1981; Ruralminas, 1996). These ecological conditions likely provided a highly stable network of temporary wetlands that accommodated the diversification of annual fish lineages. The upper rio Paracatu and upper rio Urucuia regions represent areas of remarkable biodiversity for *Hypsolebias*, where twelve species have been identified to date: six belonging to the *H. trilineatus* group, three to the *H. flammeus* group, and three to the *H. notatus* group. The high concentration of sympatric and parapatric species complexes highlights the importance of this basin for understanding the evolutionary radiation and conservation of the genus.

In conclusion, the discovery and description of *H. rosai*, *H. baptistai*, and *H. maylermartinsi* significantly expand the taxonomic and systematic understanding of *Hypsolebias* by doubling the known diversity of the newly recognized *H. trilineatus* species group and confirming the upper rio Paracatu basin as critical hotspots for annual fish diversification. Synthesizing morphological traits, such as unique male color patterns, proves essential for diagnosing species limits within this complex. However, the rapid environmental degradation driven by agricultural expansion in the northwestern Minas Gerais (Cerrado biome) threatens the survival of these endemic populations before their biology is fully understood. Future studies should focus on integrating molecular datasets to test the phylogenetic positioning of this new species group and establishing urgent, targeted conservation strategies to safeguard these highly vulnerable annual aquatic habitats.

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Márcio Joaquim da Silva: Writing-original draft, Writing-review and editing

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ETHICAL STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

AI STATEMENT

The authors did not use any AI-assisted technologies in the creation of this manuscript or its figures.

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