

Unrecognized biodiversity in a world's hotspot: three new species of *Melanorivulus* (Cyprinodontiformes: Rivulidae) from tributaries of the right bank of the Rio Paraná basin, Brazilian Cerrado

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Abstract

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The genus *Melanorivulus* presents a wide geographical distribution in the Neotropical region. Among *Melanorivulus*, the *M. pictus* species group has currently 18 species distributed in the Brazilian Cerrado, mainly in the upper Rio Paraná basin, with some species occurrences in the upper Rio Araguaia and Tocantins. In the present study, we describe three new *Melanorivulus* species, belonging to the *M. pictus* species group from different drainages of the right bank of the Rio Paraná basin in Mato Grosso do Sul state, Brazil. These new species are easily distinguished from the others by their unique colour patterns. *Melanorivulus interruptus* is distinguished from all species of the *M. pictus* group by the presence of oblique chevron-like red bars interrupted, mainly on the midline of the flank in males; while *M. ivinhemensis* by the yellow colouration of the caudal fin with thin red bars arranged only in the median region of the fin in males. *Melanorivulus amambaiensis* is distinguished from all species of the *M. pictus* species group by having an orange anal fin or sometimes more reddish-orange with distal margin grey or dark grey and chevron-like bars along the body, distinctly branched ventrally forming an inverted Y-shape in males. The high diversity of the *Melanorivulus* species with high levels of endemism demands the development of conservation strategies to avoid the loss of their vulnerable habitats in the Cerrado biome. We expect presence of more species of the *M. pictus* species group also along the lower reaches of the tributaries of the Rio Paraná. Additionally a dichotomic identification key of the *M. pictus* species group is provided.

Introduction

Savanna biomes are distributed across tropical zones around the world. In South America, the Cerrado is the largest savanna biome, the second largest biome in Brazil (smaller only than the Amazon), and represents about 23 % of Brazilian territory (Ratter et al. 1997). The Brazilian Cerrado is included in the world's 25 main “hotspots”,

areas with many rare and endemic species and less than 30% remaining natural vegetation (Myers et al. 2000). It is one of the world's most threatened biomes, mainly because of the replacement of natural vegetation by pastures and row crops (Ratter et al. 1997, Myers et al. 2000).

The rivulid genus *Melanorivulus* is a typical component of the Cerrado fauna, but presents a wide geographical distribution in the Neotropical region, being also

found in areas under the influence of the Chaco, Pantanal, Atlantic Forest and Amazon (e.g. Costa 2016, Costa et al. 2016, Nielsen 2017). However, most of the species are found in Brazil and occur in the Cerrado biome along several river basins drained mainly by the upper tributaries of the Rio Araguaia, Tocantins, Paraná and Paraguay basins and the adjacent Cerrado-Amazon ecotone (Costa et al. 2016). Recent killifish inventories of the Brazilian Cerrado have revealed a high diversity of *Melanorivulus* species (e.g., Costa 2012, 2017, 2018, Volcan et al. 2017), indicating that species inhabiting the region have small geographical ranges, often restricted to short segments of a single river drainage (Costa 2017, Volcan et al. 2017). Despite the wide geographical distribution and high diversity, little is known about the conservation, distribution, biology and ecology of most *Melanorivulus* species (Severo-Neto and Volcan 2018).

Among *Melanorivulus* species, the *M. pictus* species group is distinguished from all other congeners, by the presence of a vestigial ventral process of the angulo-articular (vs. process well-developed), curved first epibranchial (vs. approximately straight) and intense greenish blue or greenish golden to purplish blue flank above anal-fin base in males (vs. never similar colour pattern) (Costa 2007, 2008). The *Melanorivulus pictus* species group currently comprises 18 species (Volcan et al. 2017, Costa 2018), with 16 species occurring in the Rio Paraná basin: *M. apiamici* (Costa, 1989) in the Rio Paraná, *M. egeus* (Costa, 2005), in the Rio São Domingos, *M. formosensis* (Costa, 2008) and *M. nigromarginatus* Costa, 2018 for the Rio Corrente, *M. giarettai* (Costa, 2008) for the Rio Araguari basin, *M. leali* Costa, 2013 for the Rio Grande basin, *M. nigropunctatus* Volcan, Klotzel & Lanés, 2017 and *M. ofaie* Volcan, Klotzel & Lanés, 2017 for the Rio Verde basin, *M. faucireticulatus* (Costa, 2008), *M. pictus* (Costa, 1989) and *M. vittatus* (Costa, 1989) for the Rio Claro basin, *M. polychromus* Nielsen, 2016 for the Rio São José dos Dourados basin, *M. rutilicaudus* (Costa, 2005) for the Rio Verde basin, *M. scalaris* (Costa, 2005) for the Rio Sucuruí basin, *M. proximus* Costa, 2018 for Rio Aporé and *M. linearis* Costa, 2018 for the upper Rio Pardo basin. Two other species occur exclusively in the upper Rio Tocantins and Araguaia basin: *M. planaltinus* (Costa & Brasil, 2008) for the Rio Cocal and *M. litteratus* (Costa, 2005) for the Salto stream, respectively.

A previous analysis indicates that the most recent common ancestor of *Melanorivulus* probably occupied a region comprising the eastern Amazon savanna and the ecotone Amazon-Cerrado, and the current distribution is the result of a series of dispersal and vicariance events during the evolutionary history of the genus (Costa et al. 2016). In the present study, we describe three new species of *Melanorivulus* belonging to the *M. pictus* species group that are endemic to different drainages of the right bank of the Rio Paraná basin in Mato Grosso do Sul state, Brazil. Additionally, we discuss the conservation, distribution patterns, and provide an identification key to the *M. pictus* species group.

Material and methods

In December 2016, a 12-day collection campaign was conducted to sample potential habitats for the occurrence of killifish species in the Mato Grosso do Sul state, Brazil, which included the largest river basins associated with Rio Paraná. In order to define the sampling strategy, we analysed satellite images from Google Earth (earth.google.com) as well as locations of specimens vouched in the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul. Field works were undertaken travelling the main highways and roads to reach access to previously selected areas; at every site we performed an active search for fishes.

Fish samples were taken with a dip-net (D-shaped hand net, 60 cm × 40 cm, 2 mm mesh size), and then were euthanized with clove oil, fixed *in situ* with 4% formaldehyde, and later transferred into 70% ethanol. The material was collected under IBAMA/ICMBio authorization (process number 56894-1).

Morphological characters were obtained from specimens fixed in formalin after collection, and subsequently transferred to 70% ethanol. Fish measurements were taken point-to-point with digital calipers to the nearest 0.1 mm on the left side of the specimen following Costa (1995). Measurements are expressed as percentage of standard length (SL), except the head measurements, which are recorded as percentage of head length. Fin-ray counts include all elements. Scale count in the mid-longitudinal series includes all scales between the upper attachment of the opercular membrane and the base of the caudal fin, excluding small scales posterior to the hypural plate. Numbers of vertebrae were recorded only from cleared and stained specimens (C&S), prepared according to Taylor and Van Dyke (1985). Frontal squamation nomenclature follows that described by Hoedeman (1958), and for cephalic neuromasts series Costa (2001).

Descriptions of colour patterns were based on photographs of both sides of live specimens photographed in the field, which were fixed *in situ* after photos, and individuals maintained in aquaria (not preserved). Institutional abbreviations are MCP (Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre) and ZUFMS (Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul, Campo Grande).

Melanorivulus interruptus sp. n.

<http://zoobank.org/2B106159-5398-44DC-B89C-7F8CB6CE676A>

Holotype. MCP 53145, male, 32.3 mm SL, Brazil, Mato Grosso do Sul State, municipality of Campo Grande, first order stream, in the headwaters of Rio Pardo, 20°28'11"S, 54°29'25"W, altitude 589 m a.s.l.; M.V. Volcan & L.E.K. Lanés, 13 Dec 2016.

Paratypes. MCP 53146, 4 males, 21.6–31.0 mm SL (1 C&S), 3 females, 22.5–29.0 mm SL, 1 juvenile, sex undetermined, 16.9 mm, all collected with the holotype.

ZUFMS 5409, 7 females, 20.2–26.0 mm SL (3 C&S), same locality as holotype; F. Severo-Neto, T.R.F. Sinani & S. Ichikawa, 5 Aug 2017.

Diagnosis. *Melanorivulus interruptus* is distinguished from all species of the *M. pictus* group, except *M. nigromarginatus*, by the presence of oblique chevron-like bars interrupted, mainly on the midline of the flank in males (vs. bars continuous, vestigial, irregular or presence of longitudinal rows of red dots on the side of the body; never bars interrupted on the midline of the body). It is distinguished from all other species in the *M. pictus* species group, except *M. planaltinus*, *M. leali* and *M. pictus*, by the presence of a rounded dorsal fin (vs. slight pointed or pointed dorsal fin). In addition, *M. interruptus* differs from *M. amambaiensis*, *M. apiamici*, *M. egens*, *M. faucreticulatus*, *M. ivinhemensis* and *M. leali* by a higher caudal fin ray count (32–33 vs. 28–31); from *M. egens*, *M. faucreticulatus*, *M. leali*, *M. litteratus*, *M. rutilicaudus* and *M. formosensis* by the position of the anal-fin origin between the pleural ribs of 15th and 16th vertebrae (vs. 13th and 15th vertebrae); from *M. apiamici*, *M. egens*, *M. faucreticulatus*, *M. pictus*, *M. polychromus* and *M. proximus* by the dorsal-fin origin at a vertical through the base of the 8th anal-fin ray (vs. vertical through base of 7th or 9th–10th anal-fin ray); from *M. amambaiensis*, *M. egens*, *M. faucreticulatus*, *M. ivinhemensis*, *M. planaltinus*, *M. polychromus*, *M. nigropunctatus*, *M. ofaie* and *M. formosensis* by the dorsal-fin origin between neural spines of 18th and 19th vertebrae (vs. 19th and 22th); from *M. egens*, *M. faucreticulatus*, *M. leali*, *M. litteratus*, *M. scalaris*, *M. vittatus*, *M. linearis* and *M. proximus* by the tip of pelvic fin reaching the urogenital papilla in males (vs. tip of pelvic fin reaching 1st to 4th anal fin ray). Another interesting diagnostic character is the presence of 8 rays in the pelvic fin in about 35% of the analysed specimens (vs. 5–7 rays, never 8 rays present in the pelvic fin).

Description. Morphometric data are presented in Table 1. Males larger than females. Largest male examined 32.3 mm SL, largest female 29.0 mm SL. Dorsal profile slightly convex from snout to end of dorsal-fin base. Ventral profile convex from lower jaw to origin of anal-fin. Dorsal and ventral profiles of caudal peduncle nearly straight. Body slender, approximately cylindrical and compressed. Greatest body depth at pelvic-fin base. Snout weakly pointed in lateral view. Jaws short.

Short dorsal and anal fins. Dorsal-fin rays 9–10. Dorsal fin rounded in males and females. Dorsal-fin origin on vertical through base of 8th anal-fin ray, and between neural spines of 18th and 19th vertebrae. Anal-fin rays 12–13. Anal fin slightly pointed in males and females. Origin of anal fin at vertical through pleural ribs of 15th–16th vertebrae. Caudal fin rounded 32–33 rays. Pectoral fin rays 14. Pectoral fins rounded, with posterior margin reaching vertical at about 60–90% of length between pectoral-fin and pelvic-fin bases. Pelvic-fin rays 7–8. Pelvic-fin posterior tip reaching vertical at anus to 3rd anal-fin ray. Pelvic-fin bases in close proximity.

Table 1. Morphometric data for the holotype and paratypes of *Melanorivulus interruptus* sp. n.

	Holotype	Males (4)	Females (7)
Standard length	32.3	21.6–31.0	22.3–29.0
Percentages of standard length			
Body depth	25.6	20.8–24.5	21.3–26.0
Caudal peduncle depth	14.4	13.3–14.8	11.4–14.8
Predorsal length	73.3	75.0–77.1	73.3–77.9
Prepelvic length	54.6	52.6–53.9	51.8–56.3
Dorsal fin base length	11.8	11.9–13.7	10.7–12.7
Anal fin base length	21.2	18.4–22.5	17.8–19.4
Caudal fin length	28.0	26.6–32.2	26.8–29.4
Pectoral fin length	18.3	19.0–20.6	17.7–20.2
Pelvic fin length	12.1	12.6–13.9	8.5–10.4
Head length	26.2	26.8–27.5	24.9–27.7
Percentages of head length			
Head depth	77.9	66.7–78.0	66.1–76.2
Head width	76.8	66.7–73.6	74.7–82.1
Snout length	18.5	12.6–17.7	15.1–19.2
Lower jaw length	19.0	16.4–21.7	18.7–21.6
Eye diameter	28.2	28.6–31.9	31.9–35.6

Scales cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 15–25% of caudal-fin base. No scales on dorsal and anal-fin bases. Frontal squamation E-patterned; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins; transverse row of scales anterior to H-scale. Longitudinal series of scales 30–32; transverse series of scales 8–9; scale rows around caudal peduncle 16. No contact organs on flank and fins.

Cephalic neuromasts: supraorbital 3+3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1+9–11+1, preorbital 2, otic 1, postotic 1–2, supratemporal 1, median opercular 1, ventral opercular 1, preopercular 2+4, mandibular 2–3+1, lateral mandibular 1–2, paramandibular 1. Two neuromasts on caudal-fin base.

Six branchiostegal rays. Gill rakers on first branchial arch 1+7. First epibranchial slightly curved. Total number of vertebrae 29–30, 13–14 precaudal vertebrae, 16–17 caudal vertebrae. Ventral process of angulo-articular short, pointed. Vomerine teeth 1–3. Dermosphenotic present. Basihyal sub-triangular, greatest width 45–50% of length; basihyal cartilage 20–25% of total basihyal length. Second pharyngobranchial teeth absent.

Colouration in life. Males (Figs 1 and 2). Flank light grey or light metallic blue; sometimes purple-blue close to anal fin; 7–9 oblique narrow red bars anteriorly directed, often forming chevron-like marks irregularly arranged and usually interrupted in the midline of the body; commonly, the chevron-like bars are vestigial in the anterior region and begin at the end of the pectoral fin; well-defined, but interrupted, bars begin behind insertion of the pelvic fins. Inconspicuous dark pigmentation between postorbital region and area above midlength of pectoral fin, forming an irregular mid-lateral dark stripe;

vertical rows of reddish dots on antero-ventral part of flank, between pectoral and pelvic fins. Dorsum yellowish-grey, venter whitish-grey. Dorsal portion of the head yellowish-grey; ventral portion yellowish-grey or pale golden without dots, marks or any pigmentation pattern; pale golden-greenish iridescence on opercular region. Jaws dark grey or brown. Iris pale yellow to pale brown, sometimes with dark brown bars on anterior and posterior portions. Dorsal fin yellow with four or five narrow red bars. Anal fin yellow, basal portion light blue-whitish, posterior portion pale blue with two or three faint red marks; distal region becoming gradually dark red-brown. Caudal fin yellow with five to seven red oblique

parallel bars, covered generally 1/3–1/2 of caudal fin on mid-dorsal portion, sometimes red bars inconspicuous; ventral portion light yellow without bars, distal ventral region becoming gradually dark red-brown with a not well delineated margin of orange-brown colour. Pectoral fin yellowish-hyaline. Pelvic fin yellow with posterior margin orange-brown.

Females (Fig. 2). Generally flank similar to males, but with paler colours. Venter white. Dorsum and dorsal portion of head yellowish-grey; ventral portion of head yellowish-grey without spots and without any colouration pattern. Jaws yellowish-grey or brown; opercular region pale greenish-gold. Dorsal fin yellow, with three or four



Figure 1. *Melanorivulus interruptus* sp. n., MCP 53145, holotype, male, 32.3 mm SL, Campo Grande, Mato Grosso do Sul, Brazil.



Figure 2. *Melanorivulus interruptus* sp. n., MCP 53146, paratypes, living male (above) and female (below), Campo Grande city, Mato Grosso do Sul state, Brazil.

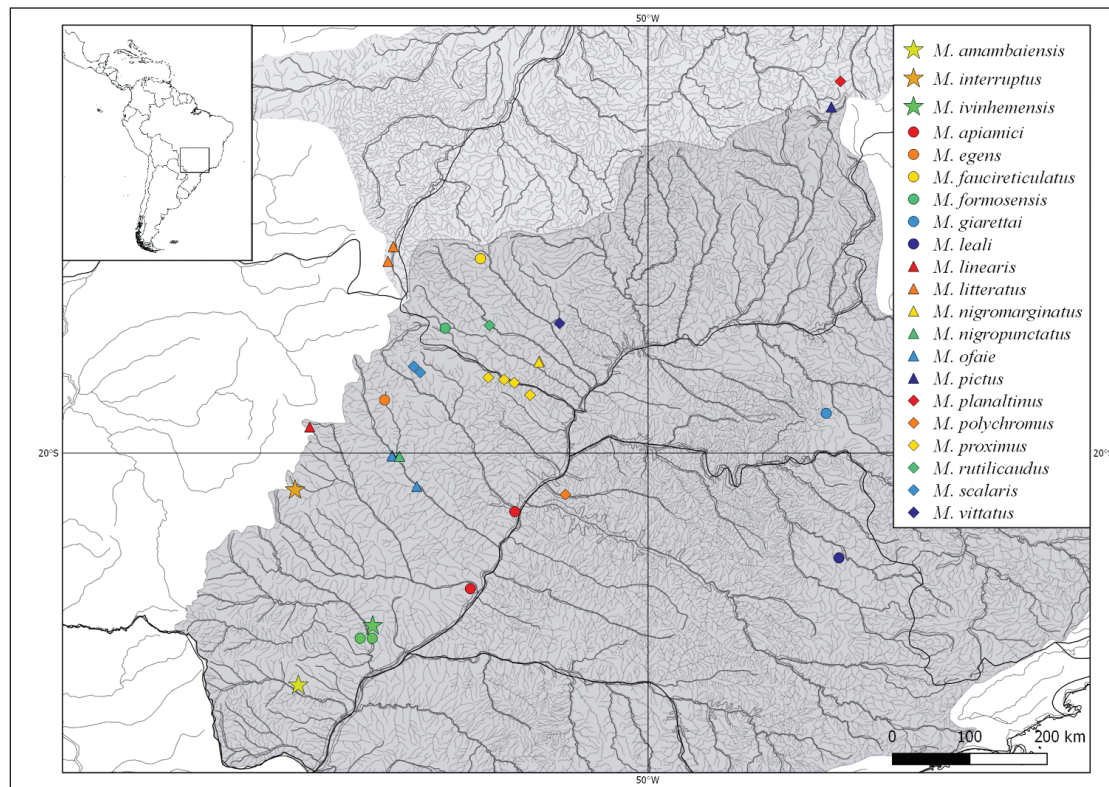


Figure 3. Distribution of species of the *Melanorivulus pictus* group in the Rio Paraná (grey) and Rio Araguaia and Tocantins (light grey) basins. Stars indicate the type localities of the new species.

faint grey bars on posterior region forming a reticulated pattern. Anal fin yellow; basal portion light blue; distal region becoming dark red-brown gradually; distal margin with high concentration of melanophores. Caudal fin pale yellow, with four or six grey bars usually interrupted in the inferior region (lower third of fin); a small irregularly shaped black spot on dorso-basal portion of fin, sometimes overlapping the first bar; faint grey margin. Pectoral fin hyaline or yellowish hyaline. Pelvic fin yellowish hyaline.

Distribution. *Melanorivulus interruptus* is only known so far from a small stream, in the headwaters of the Rio Pardo, a tributary of the right margin of Rio Paraná, state of Mato Grosso do Sul, Brazil (Fig. 3).

Etymology. The name *interruptus*, from the Latin adjective interrupted, referring to the presence of conspicuous oblique red bars forming chevron-like marks usually disrupted in the midline of the body in males.

Habitat. The species was recorded in a typical Cerrado area, inserted into the urban area of Campo Grande town at an altitude of 594 m a.s.l.. It was recorded in a small first order creek, but was only captured in border areas with dense aquatic vegetation, mainly grasses (Fig. 4). The biotope, which is locally called “veredas”, a freshwater ecosystem comprising streams bordered by the buriti-palm, typically found in the Cerrado Biome, has moderate current speed. But, the place where the species was recorded is formed by small backwaters,

with a clayey substrate and with a maximum depth of 10 cm. The species is encountered in areas exposed to the sun as well as in areas shaded by trees, mainly buriti-palm (*Mauritia flexuosa*). The region has a slightly undulating terrain and according to the classification of Koppen-Geiger has rainy summers and dry winters.

Conservation. There are imminent threats to the population of *M. interruptus*: the stream is located in the Campo Grande urban area, which is developing rapidly around the area of the new species; there is a large real estate development alongside the stream that can cause significant impacts on the environment quality; the stream is crossed by a high traffic road and it is subject to accidents with potentially dangerous loads; besides, there are agricultural activities over its complete extension. Although larger collection efforts are likely to be undertaken in the area to better understand the species distribution pattern, *M. interruptus* appears to have a very restricted distribution. In the adjacent watersheds distinct species occur (e.g. *M. apiamici* in the mouth of the Rio Pardo, *M. egens* in the upper Rio São Domingos and *M. nigropunctatus* and *M. ofaie* in the Rio Verde – see Figure 4 with the distribution map of the *M. pictus* species group). Thus, according to the previously mentioned threats, a high degree of endemism and the observations made in the field, *M. interruptus* was considered “Vulnerable”, following the criteria of IUCN (2012). The species has an area of occupation of less than 2000 km², it occurs in only one locality and it suffers from a reduction in its occupation area and in its habitat quality (VU B2abii,iii).



Figure 4. First order stream (typical “veredas” habitat), tributary of headwaters of Rio Pardo, *M. interruptus* type locality.

***Melanorivulus ivinhemensis* sp. n.**

<http://zoobank.org/2531E407-3D5B-428D-9CD3-37AB7A13600C>

Holotype. MCP 53147, male, 30.1 mm SL, Brazil, Mato Grosso do Sul State, municipality of Nova Andradina, first order stream of Rio Ivinhema, 22°11'53"S, 53°20'12"W, altitude 300 m a.s.l.; M.V. Volcan & L.E.K. Lanés, 09 Dec 2016.

Paratypes. MCP 53153, 6 males, 20.3–27.7–31.0 mm SL, 7 females, 18.5–29.7 mm SL (1 C&S), 2 juvenile, sex undetermined, 14.0–15.4 mm, all collected with the holotype. MCP 53148, 2 juvenile, sex undetermined, 14.3–16.9 mm, wetland in the boarder of BR 376 highway, 22°21'22"S, 53°30'32"W, altitude 252 m a.s.l.; M.V. Volcan & L.E.K. Lanés, 08 Dec 2016. MCP 53149, 1 male, 18.6 mm SL, 2 females, 25.7–30.4 mm SL, first order stream of Rio Ivinhema, 22°21'51"S, 53°39'42"W, altitude 310 m a.s.l.; M.V. Volcan & L.E.K. Lanés, 08 Dec 2016. ZUFMS 5365, 5 males, 21.8–29.5 mm SL (3 C&S), 3 females, 18.1–20.4 mm SL, first order stream of Rio Ivinhema, 22°12'17"S, 53°27'17"W, altitude 317 m a.s.l.; T.R.F. Sinani & M.O. Bordignon, 17 Sep 2016.

Diagnosis. *Melanorivulus ivinhemensis* is distinguished from all other species of the *M. pictus* species group by having a yellow caudal fin with thin red bars arranged only in the median region of the fin in males (vs. red bars absent or red bars extending from the ventral or median region to the dorsal region of the caudal fin), except in *M. rutilicaudus*. Besides, it is distinguished from all *M. pictus* species by the origin of the anal fin at a vertical through pleural ribs of 15th–18th vertebrae

(vs. 13–15 vertebrae), except in *M. amambaiensis*, *M. planaltinus*, *M. scalaris*, *M. polychromus*, *M. ofaie*, *M. interruptus* and *M. nigropunctatus*; and by the greater snout length in males (16.1–21.3 vs. 11.4–16.3), except in *M. amambaiensis*, *M. apiamici*, *M. interruptus*, *M. vittatus*, *M. polychromus* and *M. nigropunctatus*. Distinguished from *M. planaltinus*, *M. rutilicaudus*, *M. interruptus* by a lower caudal fin ray count (29–31 vs. 32–34) and from *M. amambaiensis* by a higher caudal fin ray count (29–31 vs. 25–28). Distinguished from *M. fau-cireticulatus*, *M. giarettai*, *M. interruptus*, *M. leali*, *M. vittatus* and *M. formosensis* by a short basihyal cartilage 10–15% of total basihyal length (vs. 20–25%). From *M. interruptus*, *M. vittatus*, *M. ofaie*, *M. nigropunctatus*, *M. proximus*, *M. linearis* and *M. nigromarginatus* it is distinguished by a smaller dorsal fin base length in females (8.9–10.4% vs. 10.5–14.8%). Distinguished from *M. giarettai*, *M. proximus*, *M. linearis*, *M. nigromarginatus* and *M. planaltinus* by lower number of gill rakers on the first branchial arch (1+7 vs 1-2+8), and from the *M. pictus* by the dorsal-fin origin on a vertical through base of 8th or 9th anal-fin ray (vs. on vertical through base of 7th anal-fin ray).

Description. Morphometric data are presented in Table 2. Females usually larger than males, largest female 30.4 mm SL, largest male examined 30.1 mm SL. Dorsal profile slightly convex from snout to end of dorsal-fin base, straight on caudal peduncle. Ventral profile convex from lower jaw to origin of anal-fin, approximately straight to end of caudal peduncle. Body slender, approximately cylindrical and compressed, greatest body depth anterior of

Table 2. Morphometric data for the holotype and paratypes of *Melanorivulus ivinhemensis* sp. n.

	Holotype	Males (n=9)	Females (n=9)
Standard length	30.1	18.6–29.5	18.5–30.4
Percentages of standard length			
Body depth	23.4	19.3–23.5	19.1–23.1
Caudal peduncle depth	14.3	12.4–14.3	11.7–14.0
Predorsal length	75.6	74.0–79.0	75.1–79.4
Prepelvic length	54.9	51.8–55.5	53.3–58.5
Dorsal fin base length	9.4	9.3–12.4	8.9–10.4
Anal fin base length	19.1	17.2–23.3	16.3–20.9
Caudal fin length	29.2	24.9–31.0	26.6–31.6
Pectoral fin length	17.1	18.4–20.8	17.5–20.7
Pelvic fin length	11.4	9.1–12.2	8.5–10.4
Head length	25.0	25.1–28.0	23.8–28.2
Percentages of head length			
Head depth	71.6	60.5–73.6	61.7–70.8
Head width	81.1	70.5–76.2	72.3–82.0
Snout length	17.3	16.1–21.3	12.9–20.6
Lower jaw length	20.6	18.3–22.0	16.8–21.6
Eye diameter	33.0	29.6–37.0	29.1–34.4

pelvic fin base, in the pelvic fin base or at the anus. Snout slightly pointed. Jaws short.

Short dorsal and anal fins. Dorsal-fin rays 7–9. Dorsal fin rounded or slightly pointed in males, rounded in females. Dorsal-fin origin on a vertical through base of 8th or 9th anal-fin ray, and between neural spines of 20th and 22th vertebrae. Anal-fin rays 12–14. Anal fin slightly pointed in males and females. Origin of anal fin at a vertical through pleural ribs of 15th–18th vertebrae. Caudal fin oval shaped, deeper than long, 29–31 rays. Pectoral fin rays 11–13. Pectoral fins rounded, with posterior margin reaching vertical at about 60–90% of the length between pectoral-fin and pelvic-fin bases. Pelvic-fin rays 6–7, one individual with one single pelvic fin. Posterior tip of pelvic fin reaching vertical between anus to 2nd anal-fin ray. Position of pelvic-fin bases variable, in close proximity, or with bases separated by a great distance, similar to the size of the base of the pelvic fin.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 15–25% of caudal-fin base. No scales on dorsal and anal-fin bases. Frontal squamation E and F-patterned or with two patterns, one on each side in one specimen. E-scales generally not overlapping medially. In one specimen E-scales marginally overlapped. Transverse row of scales anterior to H-scale; scales arranged in regular circular pattern around A-scale. A-scale usually without exposed margins, four specimens with posterior margin exposed, overlapping the B-scale. Longitudinal series of scales 29–31; transverse series of scales 8–9; scale rows around caudal peduncle 16. No contact organs on flank and fins.

Cephalic neuromasts: supraorbital 3+3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1+11–12+1, preorbital 2, otic 1, postotic 1–2, supratemporal 1, median opercular 1, ventral opercular 1–2, preopercular

2+4–6, mandibular 3–4+1, lateral mandibular 1–2, paramandibular 1. Two neuromasts on caudal-fin base.

Six branchiostegal rays. Gill rakers on first branchial arch 1+7. First epibranchial slightly curved. Total number of vertebrae 29–31, 14 precaudal vertebrae, 15–17 caudal vertebrae. Ventral process of angulo-articular short, pointed. Vomerine teeth 2–5. Dermosphenotic present. Basihyal sub-triangular, greatest width 45–50% of length; basihyal cartilage 10–15% of total basihyal length. Second pharyngobranchial teeth absent.

Colouration in life. Males (Fig. 5). Metallic greenish-gray flank, purple-blue close to anal fin; red dots on anterior flank area, sometimes few red dots on the antero-ventral region; one to three oblique narrow bars between the medial pectoral fin portion and close to the pelvic fin base, sometimes one or two of these bars forming chevron-like marks with an angle on the ventral region of the flank; 7–9 oblique narrow red bars anteriorly directed, forming chevron-like marks with an angle on midline of flank, and generally positioned posteriorly to the pelvic fin base on the caudal peduncle. Two parallel and oblique dark brown bars often interconnected by a brown-dark horizontal line between postorbital and posterior opercular region, sometimes forming a broad and well-defined mid-lateral dark stripe between postorbital region and area above the pectoral fin at mid length. Yellowish-grey dorsum with small black dots, venter white. Dorsal portion of the head yellowish-grey sometimes with small black dots; ventral portion white; golden iridescence on opercular region. Dark grey jaws varying to pale yellowish-grey. Iris pale yellow. Dorsal fin yellow with three or five oblique narrow red bars on posterior portion of fin. Anal fin yellow or orange-yellow, sometimes with a distal dark margin, basal portion light metallic blue-whitish, posterior portion pale blue with two or three faint reddish oblique bars. Caudal fin yellow or hyaline-yellowish, presenting three to six vertical or slightly oblique parallel red or red brownish bars in the central region. Pectoral fin yellowish hyaline. Pelvic fin yellowish hyaline or orange and hyaline, sometimes with a distal dark margin.

Females (Fig. 6). Flank similar to males. Dorsum and dorsal portion of head greenish-gray with small black dots; ventral portion of head white, with black marks, sometimes inconspicuous; pale golden iridescence on opercular region. Jaws pale yellowish-grey. Iris pale yellow, sometimes with dark brown bars on anterior and posterior portions. Dorsal fin yellowish-orange, with horizontally elongated dark brown to black spots or bars in the medial region; dark grey narrow margin delineating entire dorsal fin. Anal fin yellowish-orange, basal portion pale blue with two interrupted reddish bars, posterior portion pale blue with two or three reddish oblique bars, distal region becoming gradually dark reddish brown on marginal border, distal margin with high concentration of melanophores. Caudal fin orange, sometimes orange and hyaline in central portion, with three to six dark brown to black bars, sometimes bars formed by dense, vertically elongated spots, the rays and



Figure 5. Colour pattern variation of male paratypes of *M. ivinhemensis* sp. n., MCP 53153, Nova Andradina municipality, Rio Ivinhema, Mato Grosso do Sul state.

inter-spaces between them pigmented; dark brown to black circular or sub triangular spot on dorso-basal portion of the caudal fin; dark grey margin. Pectoral fins hyaline. Pelvic fins orange-yellow, with a reddish-brown anterior margin.

Distribution. *Melanorivulus ivinhemensis* is so far only known from first order streams and small wetlands associated with both margins of the lower course of the Rio Ivinhema, Rio Paraná basin (Figure 3).

Etymology. The name *ivinhemensis* is a reference to the occurrence of the new species in the Rio Ivinhema basin.

Habitat notes. *Melanorivulus ivinhemensis* was recorded in marginal areas of small first order streams and in wetlands completely exposed to the sun on a slightly undulating terrain at altitudes ranging from 267 to 315 m a.s.l. (Fig. 7). One wetland was deep (about 70 cm of maximum depth), with muddy substratum and turbid water (Fig. 7b),

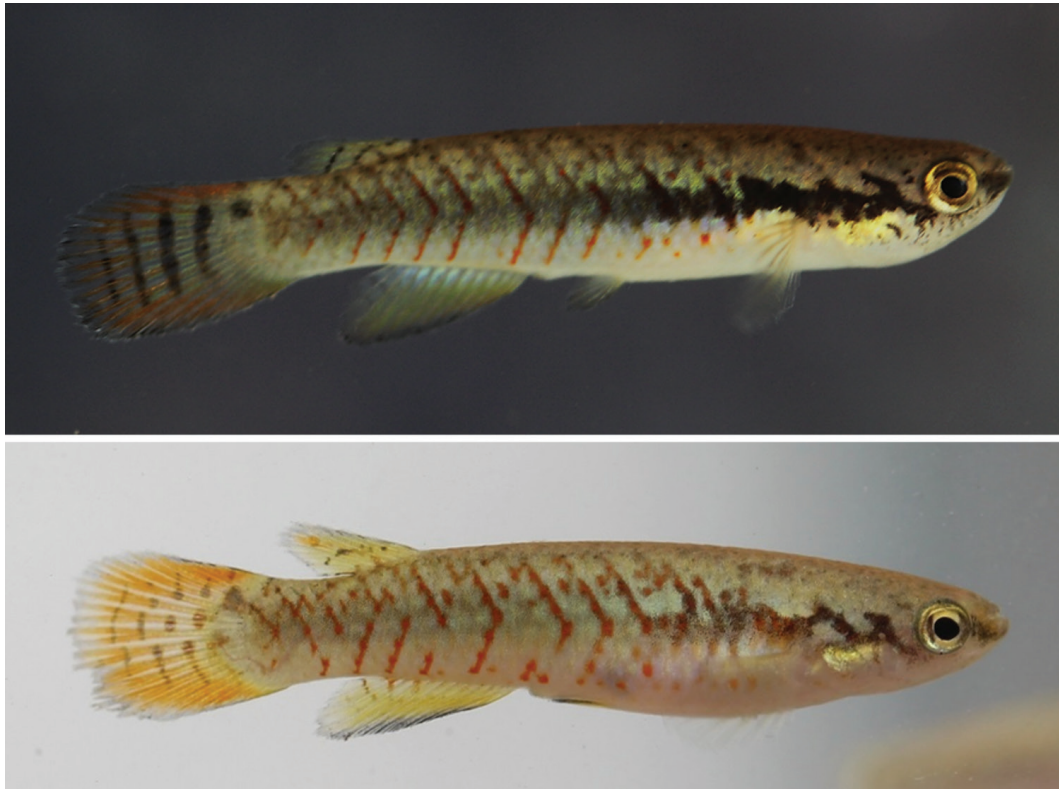


Figure 6. Female paratypes of *M. ivinhemensis* sp. n., MCP 53153, Nova Andradina municipality, Rio Ivinhema, Mato Grosso do Sul state.

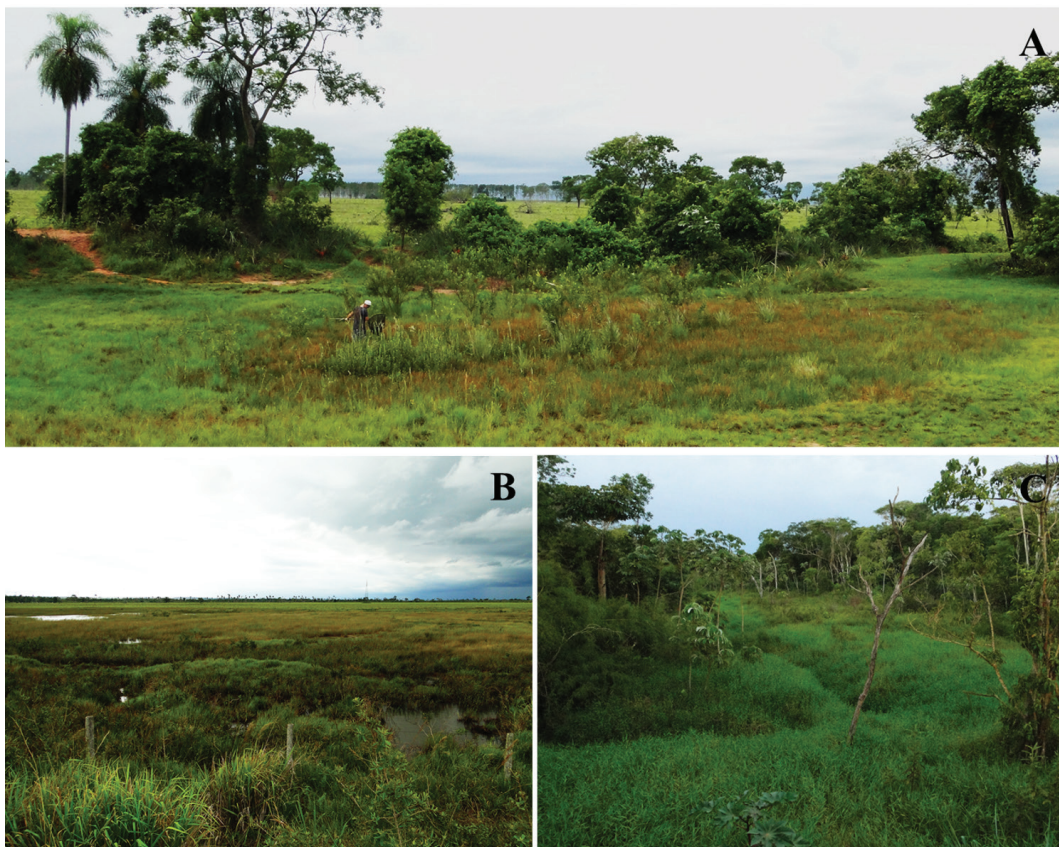


Figure 7. Sampling sites of *Melanorivulus ivinhemensis* sp. n. (a) Wetland associated with a small drainage, direct tributary of Rio Ivinhema, type locality, (b) wetland along BR 376 highway, (c) small drainage, direct tributary of Rio Ivinhema.

while in the other localities the species was recorded in shallow areas, not exceeding 20 cm depth, in crystal-line waters and with a clayey substrate (Fig 7a, c). Eight fish species were recorded co-occurring with *M. ivinhensis*: *Aphyocharax dentatus* Eigenmann & Kennedy, 1903, *Pyrrhulina australis* Eigenmann & Kennedy, 1903, *Hyphessobrycon anisitsi* (Eigenmann, 1907), *Hyphessobrycon eques* (Steindachner, 1882), *Serrapinnus kriegi* (Schindler, 1937), *Cichlasoma dimerus* (Heckel, 1840), *Hoplerethrinus unitaeniatus* (Spix & Agassiz, 1829) and *Moenkhausia sanctaefilomenae* (Steindachner, 1907).

Conservation status. The new species is endemic of Rio Ivinhema and was recorded in four different areas, in a range of about 25 km along both sides of the river and there is a large density of potential environments for its occurrence along this stretch. It has been recorded in areas with no major impacts – although under intense agricultural activity – and therefore there is no evidence that the species is threatened with extinction.

Melanorivulus amambaiensis sp. n.

<http://zoobank.org/6E2F9D08-66CB-4B41-866E-3AB0F878EFAB>

Holotype. MCP 53150, male, 27.9 mm SL, Brazil, Mato Grosso do Sul State, near of municipality of Naviraí, first order stream of Rio Amambai, margin of BR 487 highway, 22°57'11"S, 54°26'52"W, altitude 332 m a.s.l.; M.V. Volcan & L.E.K Lanés, 14 Dec 2016.

Paratypes. MCP 53151, 4 males, 20.9–25.9.0 mm SL (1 C&S), 10 females, 22.8–28.7 mm SL (3 C&S), all collected with the holotype. ZUFMS 5497, 1 male, 21.6 mm SL, 4 females, 22.2–22.9 mm SL, all collected with the holotype.

Diagnosis. The presence of an orange or orange-red anal fin with grey or dark grey distal margin (vs. no similar colour pattern) and chevron-like red bars in inverted Y-shape in the flank of males (vs. no similar pattern) distinguishes the *M. amambaiensis* from all other species of the *M. pictus* species group. Additionally, the new species is distinguished by the lower caudal fin ray count (25–28 vs. 29–34), except *M. faucireticulatus*; by the lower body depth in males (29.4–21.6 mm SL vs. 21.8–26.5), except from *M. vittatus*, *M. polychromus*, *M. nigropunctatus*, *M. interruptus* and *M. ivinhensis*; by a lower caudal peduncle depth in males (12.4–13.5 mm SL vs. 13.5–16.8 mm SL), except in *M. polychromus*, *M. nigropunctatus*, *M. interruptus* and *M. ivinhensis*. Females are distinguished from *M. apiamici*, *M. faucireticulatus*, *M. giarettai*, *M. planaltinus*, *M. rutilicaudus*, *M. scalaris*, *M. nigropunctatus*, *M. proximus*, *M. linearis*, *M. nigromarginatus* and *M. formosensis* by the lower body depth (19.1–21.5 mm SL vs. 21.6–26.0 mm SL); and from *M. apiamici*, *M. egens*, *M. faucireticulatus*, *M. giarettai*, *M. planaltinus*, *M. nigropunctatus*, *M. linearis*, *M. nigromarginatus* and *M. ofaie* by a shorter pre-dorsal length (74.5–76.6 mm SL vs. 76.4–83.3 mm SL).

Table 3. Morphometric data for the holotype and paratypes of *Melanorivulus amambaiensis* sp. n.

	Holotype	Males (n=5)	Females (n=11)
Standard length	27.9	20.9–25.9	24.0–28.7
Percentages of standard length			
Body depth	21.6	20.4–20.9	19.1–21.5
Caudal peduncle depth	12.9	12.4–13.5	11.7–13.7
Predorsal length	76.1	74.9–79.1	74.5–76.4
Prepelvic length	56.8	53.6–55.3	52.0–54.5
Dorsal fin base length	12.3	10.1–12.0	9.3–11.4
Anal fin base length	21.4	19.1–21.4	18.5–20.7
Caudal fin length	27.5	28.1–30.7	26.5–28.6
Pectoral fin length	17.1	17.9–21.0	17.3–20.1
Pelvic fin length	11.3	9.4–12.0	8.5–11.6
Head length	26.5	26.2–26.9	24.6–27.6
Percentages of head length			
Head depth	69.9	61.6–69.5	62.4–71.4
Head width	75.7	70.5–72.4	70.1–79.3
Snout length	15.2	16.7–17.8	15.2–20.6
Lower jaw length	20.6	18.3–21.3	18.0–21.0
Eye diameter	28.7	28.5–32.1	28.7–33.1

Description. Morphometric data are presented in Table 3. Females larger than males, largest female examined 28.7 mm SL, largest male 27.9 mm SL. Dorsal profile slightly convex from snout to end of dorsal-fin base, straight on caudal peduncle. Ventral profile weakly convex from lower jaw to operculum. Straight from operculum to origin of pelvic-fin and in the caudal peduncle. Body slender, cylindrical and compressed, greatest body depth at origin of pelvic-fin base. Snout blunt. Jaws short.

Short dorsal and anal fins. Dorsal-fin rays 8–9. Dorsal fin slightly pointed in males, rounded in females. Dorsal-fin origin on a vertical through base of 8th or 9th anal fin ray, and between neural spines of the 20th and 21st vertebrae. Anal-fin rays 12–14. Anal fin slightly pointed in males and females. Origin of anal fin at a vertical through pleural ribs of 15th–16th vertebrae. Caudal fin oval shaped, longer than deep, 25–28 rays. Pectoral fin rays 11–13. Pectoral fins rounded, with posterior margin reaching vertical at about 70–90% of length between pectoral-fin and pelvic-fin bases. Pelvic-fin rays 6–7. Posterior tip of pelvic fin reaching a vertical at slightly anterior to the anus to 1st anal-fin ray. Pelvic-fin bases in close proximity.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of the head. Body squamation extending over anterior 15–20% of caudal-fin base. No scales on dorsal and anal-fin bases. Frontal squamation E and F-patterned or with two patterns, one on each side (present in one specimen). E-scales not overlapping medially. Transverse row of scales anterior to H-scale; scales arranged in regular circular pattern around A-scale. A-scale without exposed margins. Longitudinal series of scales 27–31; transverse series of scales 8–9; scale rows around caudal peduncle 16. No contact organs on flank and fins.

Cephalic neuromasts: supraorbital 3+3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1+9–11+1–2, preorbital 2, otic 1, postotic 1–2, supratemporal 1,



Figure 8. Colour pattern variation of male paratypes of *M. amambaiensis* sp. n., MCP 53151, small stream tributary of Rio Amambai, Mato Grosso do Sul state.

median opercular 1, ventral opercular 1–2, preopercular 2+4–5, mandibular 2–3+1, lateral mandibular 1–2, paramandibular 1. Two neuromasts on caudal-fin base.

Six branchiostegal rays. Gill rakers on first branchial arch 1+7–8. First epibranchial slightly curved. Total number of vertebrae 30–31, 13–14 precaudal vertebrae, 17 caudal vertebrae. Ventral process of angulo-articular short, pointed. Vomerine teeth 2–3. Dermosphenotic present. Second pharyngobranchial teeth absent.

Colouration in life. Males (Fig. 8). Flank light metallic blue-whitish, sometimes purplish above anal fin; numerous oblique narrow red bars irregularly arranged, forming reticulated chevron-like marks anteriorly directed with a branching on the lower portion in inverted Y-shape, sometimes with a branching on the upper and lower portions in X-shape; between these bars one to four red dots oblique-

ly distributed mostly on dorsal portion of flank; reddish dots on anteroventral part of flank, sometimes forming rows, mostly between bases of the pectoral and the pelvic fin; one to three oblique reddish-brown bars between the postorbital region and the pectoral fin; reddish-brown spots on the humeral region. Dorsum metallic grey or yellowish grey, venter white. Dorsal portion of head yellowish-grey; ventral portion white, without marks or any pigmentation pattern; golden iridescence on opercular region. Jaws grey to pale yellowish-grey. Iris pale yellow. Dorsal fin yellowish-hyaline with three or four oblique narrow brownish-red or red bars; basal portion pale yellow. Anal fin orange or orange-red, basal portion pale blue with reddish dots forming reticulated pattern, posterior portion pale blue sometimes with two or three reddish oblique bars, distal region grey or dark grey on the marginal border. Caudal fin hyaline or yellowish-hy-



Figure 9. Colour pattern variation of females of *M. amambaiensis* (not preserved), sp. n., small stream tributary of Rio Amambai, Mato Grosso do Sul state.

aline, yellowish-orange on ventral portion; four to seven narrow oblique brownish-red or red bars cover 2/3 of caudal fin, absent in ventral portion. Pectoral fin hyaline, or yellowish-hyaline. Pelvic fin orange or yellowish-orange, sometimes with orange-brown anterior margin.

Females (Fig. 9). Flank similar to males, but more evident reddish-brown or brown spots between pectoral and pelvic fins. Numerous oblique narrow red bars irregularly arranged, forming reticulated chevron-like marks anteriorly directed, sometimes with a branching on the lower portion in inverted Y-shape. Dorsum and dorsal portion of head greenish-gray with small black dots; ventral portion of head white, with dark grey spots; golden iridescence on opercular region. Jaws greyish-brown. Iris pale yellow, sometimes bordered with grey. Dorsal fin yellowish-orange or yellowish hyaline, with three reddish-brown narrow horizontal bars forming a reticulated pattern. Anal fin orange-yellow, basal portion pale blue with reddish dots forming a reticulated pattern, posterior portion pale blue with two or three reddish oblique bars, distal region becoming gradually dark reddish brown on the marginal border. Caudal fin yellowish-orange, with three to seven grey or dark grey bars; small elongated black spot on dorso-basal portion of the fin, sometimes overlapping the most anterior bar. Pectoral fins hyaline. Pelvic fins yellowish-orange, sometimes with reddish-brown anterior margin.

Distribution. The species is only known from its type locality, a small first order drainage and direct tributary of Rio Amambai, state of Mato Grosso do Sul, Brazil (Fig. 3).

Etymology. The name *amambaiensis* is a reference to the occurrence of the new species in the Rio Amambai basin.

Habitat notes. *Melanorivulus amambaiensis* was recorded in a small natural drainage, direct tributary of the Rio Amambai, with depth not exceeding 50 cm and with low flow in an area totally exposed to the sun, parallel to BR 487 highway (Fig. 10). The region has a smoothly undulating terrain and the area of occurrence is at an altitude of 327 m a.s.l.. The drainage has a clayey substrate, which gives an orange colour to the water when disturbed. Only *Gymnotus* aff. *carapo* Linnaeus, 1758 was registered co-occurring with *M. amambaiensis*.

Conservation status. The species is known only from its type locality. It seems to be a micro endemic of the Rio Amambai basin, since other species occur in the adjacent basins. Part of its original area was fragmented by BR 487 and there is high frequency traffic of vehicles on this highway. Consequently, the species is subject to accidents with potentially dangerous loads, which present a threat to the population of *M. amambaiensis*. In addition, the surrounding region is largely degraded by agricultural activities that also threaten its habitat quality. Thus, *M. amambaiensis* was accounted as “Vulnerable” according to the IUCN (2012) standards. The species has an area of occurrence of less than 2000 km², occurs in only one locality and suffers from a reduction in its area of occupation and in the quality of its habitat (VU B2abii, iii).

Key to *Melanorivulus pictus* species group from the Rio Paraná, Tocantins and Araguaia basin.

- 1 Red pigmentation forming chevron-like bars in the body of males 2
- Red pigmentation in the flank arranged in irregular lines to form vermiculate color pattern in males *Melanorivulus giarettai*
- 2 Pelvic fin present, 1–5 vomerine teeth 3
- Absence or extreme reduction of pelvic fin, 5–7 vomerine teeth *Melanorivulus planaltinus*
- 3 5 rays in the pelvic fin, 10 rays in the pectoral fin, 27 vertebrae *Melanorivulus apiamici*
- 6–8 rays in the pelvic fin, 11–14 rays in the pectoral fin, 29–32 vertebrae 4
- 4 Red bars present in the caudal fin 5
- Red bars absence in the caudal fin *Melanorivulus egens*
- 5 25–28 caudal fin ray, anal fin orange or orange-red in males *Melanorivulus amambaiensis*
- 28–34 caudal fin ray, anal fin yellow, light yellow to orange or yellowish-hyaline in males 6
- 6 7 scales in transversal series, oblique rows of red dots on flank, more concentrated on caudal peduncle, forming chevron-like marks *Melanorivulus leali*
- 8–10 scales in transversal series, oblique red bars forming chevron-like bars along the body, with forward-pointing vertex, generally in the posterior portion of the body 7
- 7 Chevron-like red bars interrupted on midline of flank, fragmented or vestigial in the body of adult males 8
- Chevron-like red bars well defined, generally not interrupted in the body of males 10
- 8 Dorsal fin rounded in males, 14 pectoral fin rays *Melanorivulus interruptus*
- Dorsal fin pointed or slightly pointed in males, 12–13 pectoral fin rays 9
- 9 Frontal squamation E-patterned, pectoral fin hyaline and pelvic fin light yellow in males... *Melanorivulus nigromarginatus*
- Frontal squamation F-patterned, pectoral and pelvic fin orange in males *Melanorivulus nigropunctatus*
- 10 Red bars present only on middle of caudal fin in males 11
- Caudal fin with dorsal and middle portions presenting vertical red bars 12
- 11 10–12 dorsal fin rays, 32–34 caudal fin rays, 34–35 scales in longitudinal series, 10 scales in transversal series *Melanorivulus rutilicaudus*
- 7–9 dorsal fin rays, 29–31 caudal fin rays, 29–31 scales in longitudinal series, 8–9 scales in transversal series *Melanorivulus ivinhemensis*
- 12 Dorsal fin origin on vertical of 7th ray of anal fin, 11–12 anal fin ray, dorsal fin rounded *Melanorivulus pictus*
- Dorsal fin origin on vertical of 7–10th ray of anal fin, 12–14 anal fin ray, dorsal fin pointed or slightly pointed 13
- 13 6 pelvic fin rays, dorsal fin origin between neural spines of vertebrae 21th–22th, absence of red color in dorsal fin *Melanorivulus polychromus*
- 6–7 pelvic fin rays, dorsal fin origin between neural spines of vertebrae 18th–21th, red pigmentation present in dorsal fin 14
- 14 Frontal squamation E-patterned 15
- Frontal squamation F-patterned *Melanorivulus litteratus*
- 15 Broad sub-basal red strip on dorsal fin in males, black reticulate pattern in ventral portion of the head of females, 28–30 caudal fin rays *Melanorivulus faucireticulatus*
- No distinctive broad sub-basal red strip on dorsal fin in males, sometimes black pigmentation present in ventral portion of the head of females never forming an reticulate pattern, 30–34 caudal fin rays 16
- 16 One gill raker on upper limb of first branchial arch 17
- Two gill rakers on upper limb of first branchial arch *Melanorivulus proximus*
- 17 Red dots present on the anteroventral portion of flank, distinctive dark marks on humeral region in males 18
- Red dots absent on the anteroventral portion of flank, absence of dark marks on humeral region in males *Melanorivulus linearis*
- 18 Flank greenish blue to metallic blue, with oblique red bars forming narrow chevron-like bars, posterior tip of pelvic-fin reaching vertical at anus to 1st anal-fin ray 19
- Flank intense metallic blue with broad red chevron-like oblique bars, posterior tip of pelvic-fin reaching vertical at 1st to 4th anal-fin ray 20
- 19 8 scales in transversal series, origin of anal fin at vertical through pleural ribs of 14th–15th vertebrae, chevron-like red bars with a branching on the upper portion forming Y-shaped red marks in males *Melanorivulus formosensis*
- 9–10 scales in transversal series, origin of anal fin at vertical through pleural ribs of 15th–16th vertebrae, chevron-like red bars in males never forming Y-shaped red marks *Melanorivulus ofaie*
- 20 Small red squares in the flank aligned to form broad red chevron-like oblique bars in males, 12–13 pectoral fin rays, 2 vomerine teeth *Melanorivulus scalaris*
- Broad chevron-like oblique red bars in males not forming squares, 14 pectoral fin rays, 3–4 vomerine teeth *Melanorivulus vittatus*



Figure 10. Water source of a first-order stream on the left bank of the Rio Amambaí, marginally situated along the BR 487 highway, type locality of *M. amambaiensis*.

Discussion

Relationships among species belonging to the *M. pictus* group are not clear. Usually there is a high overlap in meristic, morphometric and osteological characters among

congeners, however with well defined, homogeneous and structured colour patterns in each species (Fig. 11). The relatively low variability of character states among species of the *M. pictus* group makes colour pattern characters an essential source to diagnose species and to es-

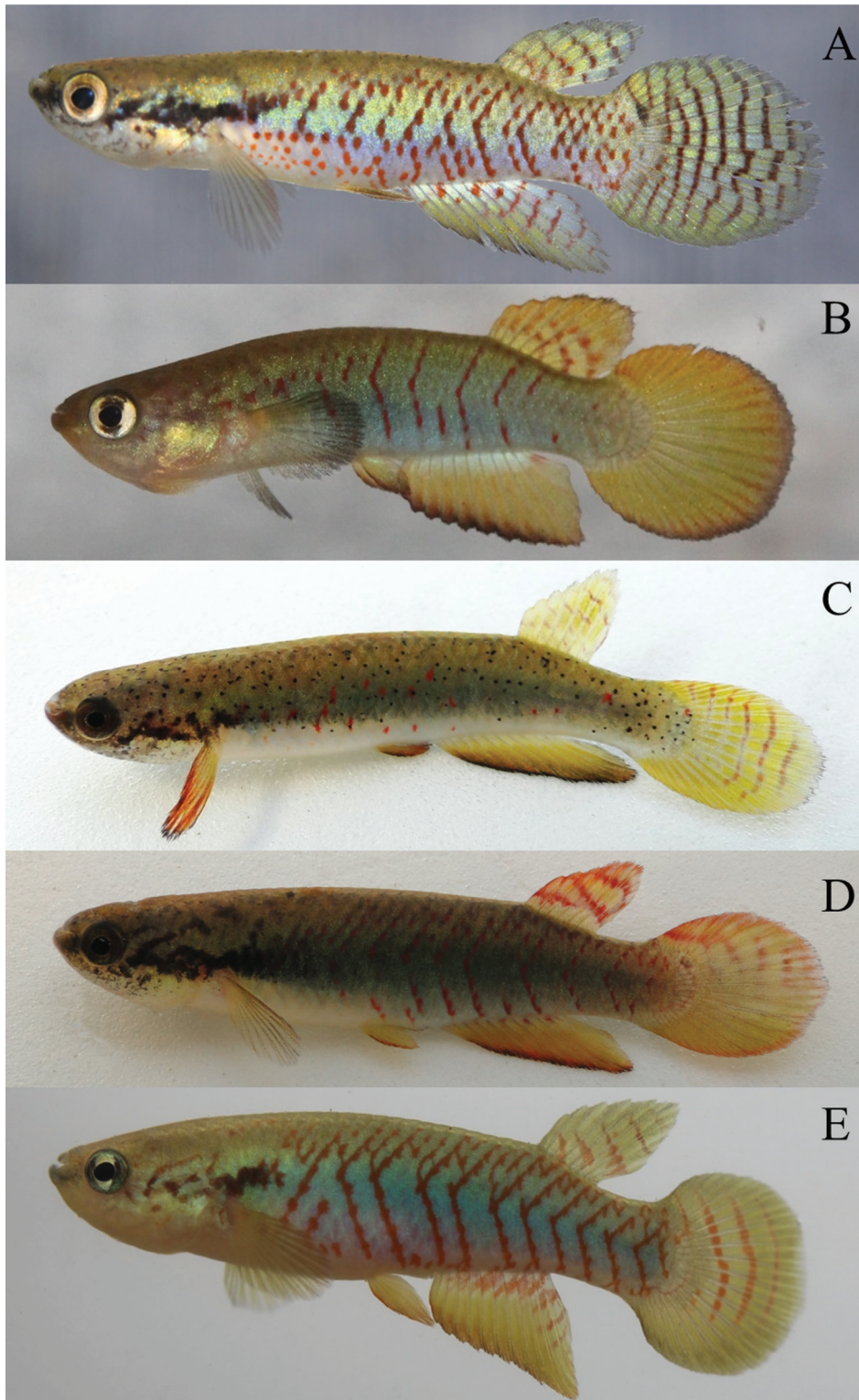


Figure 11. Males of different species of the *M. pictus* group recorded in the region of the right bank of Rio Paraná. **A)** *M. apiamici*; **B)** *M. egens*; **C)** *M. nigropunctatus*; **D)** *M. ofaie*; **E)** *M. scalaris*. Photos by Matheus Volcan.

timate their relationships, since molecular data are still not available for most species (Costa, 2018). The only molecular study corroborates species and groups delimitations according to colour patterns (Costa et al. 2016).

Costa (2016) discussed the importance of using live colour pattern characters to diagnose species and species groups of *Melanorivulus*. According to Costa (2016, 2017) patterns involving the caudal fin contain a high

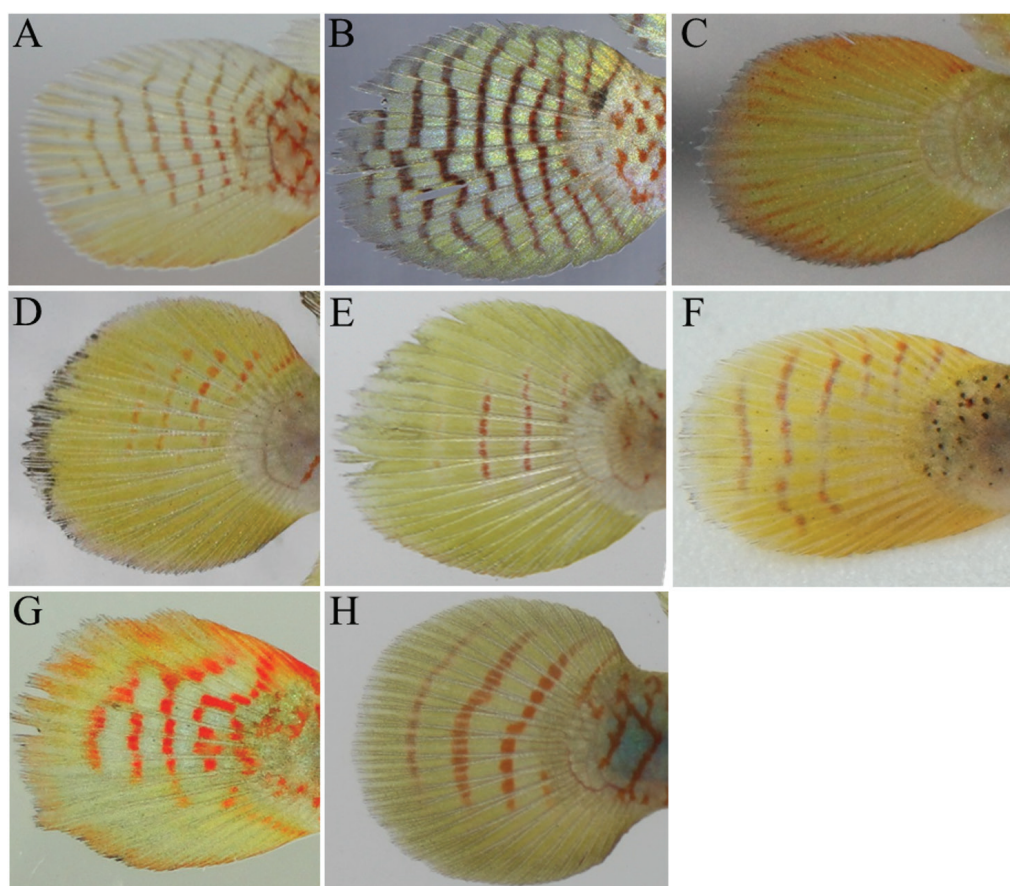


Figure 12. Variation of colour pattern of the caudal fin of males of different species of the *Melanorivulus pictus* group from Rio Paraná, Brazil. **A)** *M. amambaiensis*; **B)** *M. apiamici*; **C)** *M. egens*; **D)** *M. interruptus*; **E)** *M. ivinhemensis*; **F)** *M. nigropunctatus*; **G)** *M. ofaie*; **H)** *M. scalaris*.

concentration of phylogenetically informative characters, useful to delimit most species of the *M. zygonectes*, *M. pinima* and *M. dapazi* group. The same is also valid to distinguish species of the *M. pictus* group (Costa, 2018).

The three new species present distinct and unique colour patterns, mainly in relation to the pigment and arrangement of the chevron-like bars along the body, the colour pattern of the fins, as well as the position and shape of the bars in the caudal fin. Males of *M. ivinhemensis*, for example, have a yellow caudal fin, with vertical bars arranged only in the median portion of the fin, *M. interruptus* has a yellow caudal fin with diagonal red bars, sometimes vestigial and inconspicuous, extending from the median to the dorsal region, while males of *M. amambaiensis* have a hyaline or yellowish-hyaline caudal fin with yellowish-orange ventral portion and irregular brownish-red bars. This is always combined with an orange or orange-red anal fin with a dark grey margin (Fig. 12). Likewise, the colour and the arrangement pattern of vertical bars on the caudal fin, as well as the black spot in the caudal peduncle are highly variable in shape and size among females of the different species of the *M. pictus* species group (Fig. 13) and also are informative for species identification.

It is hypothesized that *M. interruptus* is closely related to *M. linearis* as judged by the absence of distinctive dark

marks on the humeral region, and the absence of red dots on the anteroventral portion of the flank. With *M. egens* it shares the E-pattern of the frontal squamation, the yellow unpaired fins and the absence or reduction of red bars or red pigmentation at the base of the anal fin in males. Females of these three species also present all unpaired fins yellow with a dark grey distal margin as well as hyaline pectoral fins.

Melanorivulus ivinhemensis shares some characteristics with *M. rutilicaudus* and seems to be more closely related to this species. Mainly this is because both have the unpaired fins coloured yellow and red bars are present only in the middle of the caudal fin in males, a unique pattern among the species of the *M. pictus* species group. On the other hand, relationships between *M. amambaiensis* and the other species of the *M. pictus* species group are not clear because of the unique colour pattern. Chevron-like red bars with inverted Y-shaped red marks and an orange or orange-red anal fin with a dark grey distal margin in males are not encountered in any other species of the group.

Species of the *M. pictus* group generally present a high degree of endemism, occurring in small stretches of basins that drain the upper Rio Paraná and the upper Rio Araguaia and Tocantins (See Figure 3 distribution map of *M. pictus* species group) and are known for a few sites only (e.g. Costa 2005, 2007, 2008, 2018, Volcan et al.

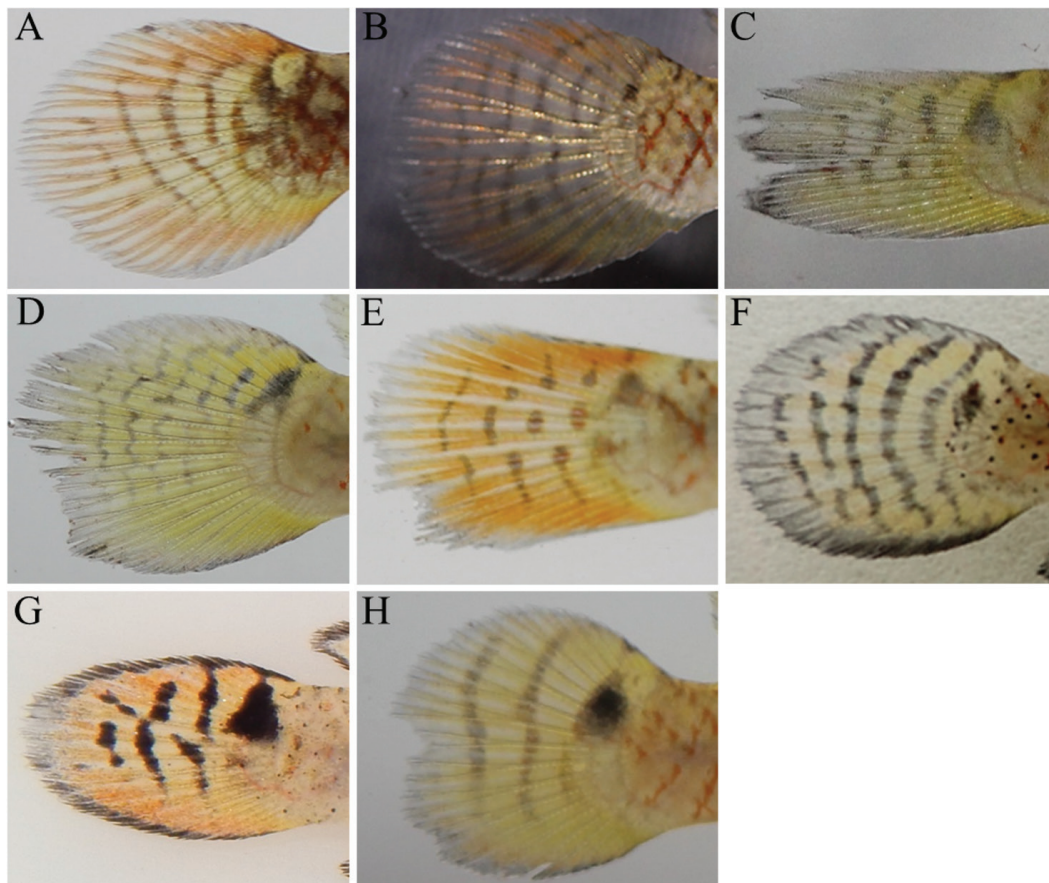


Figure 13. Variation of colour pattern of the caudal fin of females of different species of the *Melanorivulus pictus* species group from Rio Paraná, Brazil. **A)** *M. amambaiensis*; **B)** *M. apiamici*; **C)** *M. egens*; **D)** *M. interruptus*; **E)** *M. ivinhemensis*; **F)** *M. nigropunctatus*; **G)** *M. ofaie*; **H)** *M. scalaris*.

2017). This pattern is observed for different *Melanorivulus* species groups (e.g. clade *M. zygonectes* and *M. dapazi*), where species are restricted to small portions of hydrographic basins in a similar way (Costa 2016, 2017). This micro endemism is extremely concerning from the conservation perspective. The loss and/or degradation of a small stretch of a river basin may result in the extinction of a species, as in the case of the vulnerable populations of *M. amambaiensis* and *M. interruptus*, which are known only from their type localities.

Most species of the *M. pictus* group are reported for highlands, mainly in the headwaters of the main tributary drainage on the right bank of the Rio Paraná (Costa 2012, 2018, Volcan et al. 2017). On the other hand, perhaps caused by insufficient sampling, there is a large gap of records in lower areas, downstream the headwaters (Figure 3). Here, only *M. apiamici* is recorded, in lower areas associated to the main channel of the Rio Paraná. From the availability of potentially suitable habitats, we would expect the presence of representatives of the *Melanorivulus pictus* species group also along the lower reaches of most of the tributaries of the Rio Paraná.

Costa (2017) suggests that the high species diversity in the Cerrado is correlated with the topography, which generated geographical isolation of populations and may

explain the present distribution of distinct species of *Melanorivulus* along different altitudinal zones of river drainages. However, in addition to topography, it is observed that many of the species are restricted to small watersheds, sometimes in similar ranges of altitude, as in the case of *M. linearis* and *M. interruptus* that both are recorded in the headwaters of the Rio Pardo or *M. nigropunctatus* and *M. ofaie*, both occur on opposite sides of the Rio Verde. Thus, besides the topography, micro watersheds can also constitute barriers, suggesting that their restricted areas of distribution are a consequence of the low vagility of these species. We can assume a similar situation of high diversity and putative undiscovered species in other areas and basin tributaries of the upper Rio Paraná. These drain large areas of the Cerrado, mainly downstream from the headwaters of the main drainage formations of the upper Rio Paraná, where there is insufficient sampling of rivulid fish.

Comparative material

All from Brazil, Mato Grosso do Sul state. *Melanorivulus nigropunctatus*: Holotype. MCP 50017, male, 24.1 mm SL, stream tributary of left margin of Rio Verde,

20°02'29"S, 53°9'42"W; B. Klotzel, 29 Dec 2013. Paratypes. MCP 50018, 5 males, 17.9–23.6 mm SL (1 C&S), 2 females, 20.7–22.7 mm SL (1 C&S), all collected with the holotype. *Melanorivulus ofaie*: Holotype. MCP 50019, male, 30.5 mm SL, stream tributary of right margin of Rio Verde, 20°25'27"S, 52°56'34"W; B. Klotzel, 29 Dec 2013. Paratypes. MCP 50022, 5 male, 19.9 mm SL (1 C&S), 6 females, 17.3–24.3 mm SL (2 C&S), collected with the holotype. MCP 50020, 3 males, 17.2–23.3 mm SL, 4 females, 16.2–19.3 mm SL, stream tributary of right margin of Rio Verde, 20°01'50"S, 53°15'14"W.; B. Klotzel, 11 Jul 2013. MCP 50021, 4 juveniles, sex undetermined, 12.8–15.6 mm SL, stream tributary of right margin of Rio Verde, 20°02'11"S, 53°15'26"W; B. Klotzel, 11 Jul 2013. ZUFMS-PIS 4736, 4 male, 21.5–23.3 mm SL, 3 females, 17.3–20.1 mm SL. same locality as holotype; B. Klotzel, 11 Jul 2013. *Melanorivulus apiamici*: ZUFMS 5500, 4 males 20.2–24.2 mm SL, 1 female, 20.8 mm SL, topotypes, Bataguassu, 21°43'32"S, 52°15'41"W; M.V. Volcan & L.E.K. Lanés, 12 Dec 2016. *Melanorivulus egens*: MCP 53152 3 males 17.8–20.6 mm SL, 1 female, 17.5 mm SL, topotypes, Camapuã, 19°19'25"S, 53°21'06"W; M.V. Volcan & L.E.K. Lanés, 12 Dec 2016. *Melanorivulus rossoi*: ZUFMS PIS 5431, 5 males 18.1–21.0 mm SL, 4 females, 18.5–19.8 mm SL, Campo Grande, 20°40'08"S, 54°45'20"W; F. Severo-Neto, 18 Jun 2015. *Melanorivulus scalaris*: ZUFMS 2114, 6 males 14.1–23.6 mm SL, 3 females, 17.7–22.1 mm SL, topotypes, Costa Rica, 18°53'57"S, 52°58'04"W; O. Froehlich, M. Casaro, N.C Penatti & L.S. Inocêncio, 31 Mar 2004. *Melanorivulus punctatus*: ZUFMS 5254, 5 males, 17.5–18.9 mm SL, 5 females, 16.1–17.8 mm SL, Corumbá, 19°34'36"S, 57°01'10"W; F. Severo-Neto, 17 Dec 2015.

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