Three new species of the killifish genus *Melanorivulus* from the central Brazilian Cerrado savanna (Cyprinodontiformes, Aplocheilidae)

Wilson J. E. M. Costa

*Laboratory of Systematics and Evolution of Teleost Fishes, Institute of Biology, Federal University of Rio de Janeiro, Caixa Postal 68049, CEP 21941-971, Rio de Janeiro, Brazil*

Corresponding author: Wilson J. E. M. Costa (wcosta@acd.ufrj.br)

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Abstract

Three new species are described from the Neotropical region comprising the Cerrado savannas of the central Brazilian plateaus, which is among the most important biodiversity centres in the world. These species are considered closely related to *M. dapazi* from the same region, with which they share the presence of a rudimentary interarcual cartilage and a dark reddish brown distal margin on the male anal fin. The group comprising *M. dapazi* and the three new species is here named as the *M. dapazi* species group. *Melanorivulus ignescens* sp. n., from the upper Rio Araguaia basin, is distinguished from all other species of the *M. dapazi* group by the anal-fin colour pattern in males; *M. flavipinnis* sp. n. and *M. regularis* sp. n. from the Rio Paraguai basin are distinguished from all other congeners of the *M. dapazi* group by the colour pattern of the caudal fin and number of scales in the longitudinal series, respectively. All the new species are further unambiguously diagnosed by unique combinations of morphological characters, including meristic and morphometric data, and colour patterns. This study reinforces the importance of using live colour patterns to diagnose species and species groups of the genus *Melanorivulus*, but also indicates that osteological characters may be informative for species diagnosis. This study confirms the high diversity of species of *Melanorivulus* in the central Brazilian Cerrado plateaus already reported in previous studies, indicating that endemic species are often restricted to short segments of a single river drainage.

Keywords

Biodiversity hotspot, morphology, osteology, systematics, taxonomy
Introduction

The region comprising the Cerrado savannas of central Brazil has been considered among the most important biodiversity hotspots in the world (Myers et al. 2000), although many organisms endemic to this region were insufficiently sampled and poorly known until recent years (Costa et al. 2016). A typical component of the Cerrado fauna is the killifish genus *Melanorivulus* Costa, 2006, with species inhabiting the Veredas, a Cerrado ecosystem consisting of small streams running in shallow valleys, often exhibiting the buriti-palm *Mauritia flexuosa* along their banks (e.g., Costa 2007a; Oliveira et al. 2012). Probably as a consequence of small size, usually not surpassing 45 mm of total length, species of *Melanorivulus* occurring in this ecosystem were not represented in collections until recent years, with the great majority of the approximately 40 species occurring in the central Brazilian Cerrado being described only after 2005 (e.g., Costa 2012; Costa et al. 2016).

The greatest diversity among species of *Melanorivulus* endemic to the Cerrado is concentrated in the central-western Brazilian plateaus, which range in altitudes from 400 to 1,100 m above sea level (asl), in the Caiapó mountain range (Costa 2012). This area is drained by the upper tributaries of the Rio Araguaia, flowing north and belonging to the Amazonas–Tocantins river system, and the upper Paraguai and Paraná river basins, flowing southwest and south, respectively, and belonging to the Paraná–Paraguay–Uruguay river system. A total of 12 species have been recorded for this area, of which four are endemic to the Araguaia basin, one to the Paraguai basin, and seven to the Paraná basin (Costa 1989, 2005, 2006a, 2007a–b, 2008, 2012). During a recent expedition to this area, three new species were collected, one from the upper Araguaia basin and two from the Paraguai basin. All the three new species are considered to be closely related to *M. dapazi*, endemic to the Paraguai basin, by all sharing a rudimentary interarcual cartilage and a dark reddish brown stripe on the distal margin of the anal fin in males (vs. interarcual cartilage well-developed and never a similar stripe on the anal-fin distal margin; see Discussion below). This assemblage is hereafter called the *M. dapazi* species group and the three new species are herein described.

Material and methods

Specimens were captured with small dip nets (40 × 30 cm) and were euthanized soon after collection. Representative live specimens were kept alive for nearly 24 hours, photographed, and then euthanized. Euthanasia was conducted in a buffered solution of tricaine methanesulfonate (MS-222) at a concentration of 250 mg/l, for a period of about 10 minutes, i.e., until opercular movements ceased. Specimens were fixed in formalin for a period of 10 days, and then transferred to 70% ethanol. Collections were made with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) and methods for euthanasia were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit
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number: 01200.001568/2013-87). Material is deposited in Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ) and Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão, Chapadinha (CICCAA).

Descriptions of colouration of living fish were based on photographs of both sides of individuals. Photographs were taken in small aquaria around 24 hours after collection. Additional direct observations were made with fish in small transparent plastic bottles just after collection. Measurements and counts follow Costa (1988). Measurements are presented as percentages of standard length (SL), except for those related to head morphology, which are expressed as percentages of head length. Fin-ray counts include all elements. Four specimens, two males and two males, were cleared and stained for osteological analysis using the methods presented in Taylor and Van Dyke (1985); the abbreviation C&S in lists of material indicates those specimens that were prepared for osteological examination. Terminology for osteological structures followed Costa (2006b), for frontal squamation Hoedeman (1958), and for cephalic neuromast series Costa (2001). Osteological characters used in species descriptions are those that show variability within Melanorivulus (e.g., Costa 2016). Herein, geographical localities involved terms popularly adopted in the local region to compose names of geographical accidents (e.g., rio, ribeirão) allowing more accurate identifications of localities in the field and avoiding common mistakes when tentatively translating them to English; following this reasoning, Rio Paraguai is used instead of Paraguay River. New species descriptions are listed according to their type localities, from north to south.

Results

Melanorivulus ignescens sp. n.
http://zoobank.org/01008AFD-8842-4DED-8E5F-4D7DF54463F9
Figs 1–2, Table 1

Holotype. UFRJ 6875, male, 27.7 mm SL; Brazil: Mato Grosso state: Guiratinga municipality: stream tributary to Rio Bandeira, Rio das Garças drainage, Rio Araguaia drainage, 16°21'54"S, 53°47'58"W, altitude approximately 520 m asl, road MT-270, approximately 3 km southwest of the village of Guiratinga; W. J. E. M. Costa et al., 11 August 2016.

Paratypes. UFRJ 6876, 13 males, 15.8–25.5 mm SL, 18 females, 17.7–23.4 mm SL; UFRJ 6877, 2 males, 24.0–25.1 mm SL, 2 females, 22.4–23.4 mm SL (C&S); CICCAA00277, 1 male, 20.6 mm SL, 1 female, 18.6 mm SL; collected with holotype.

Diagnosis. Melanorivulus ignescens is distinguished from all other species of the M. dapazi group by having the anal fin, in adult males, bright reddish orange (vs. yellow in M. dapazi, M. flavipinnis, and M. regularis). Also distinguished from all other congeners of the M. dapazi group by the following combination of character states: 5–6 pelvic-fin rays (vs. 7 in M. dapazi and M. regularis); 29–31 scales in longitudinal series (vs. 35–37 in M. regularis); female caudal spot inconspicuous in live fish (vs. conspicuous in M.
Figure 1. *Melanorivulus ignescens* sp. n., holotype, UFRJ 6875, male, 27.7 mm SL. Photograph by W.J.E.M. Costa.

dapazi and *M. regularis*); caudal fin, in males, without red bars and distinctive orange margin (vs. with red bars in *M. regularis* and *M. flavipinnis*, with broad bright orange band along the whole margin in *M. dapazi*); in females, ventral surface of the head with dark grey spots, often forming short stripe on the chin (vs. without dark grey spots in *M. dapazi*); caudal-fin short, its length 26.8–33.1% SL (vs. long, its length 34.1–38.7% SL in *M. flavipinnis*). Also distinguished from all other species of the *M. dapazi* group by having a constriction on the metapterygoid (vs. constriction absent).

**Description.** Morphometric data appear in Table 1. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth at vertical just in front of pelvic-fin base. Dorsal and ventral profiles of trunk almost straight to slightly convex in lateral view; dorsal and ventral profiles of caudal peduncle nearly straight. Head moderately wide, sub-triangular in lateral view, dorsal profile nearly straight, ventral profile convex. Jaws short, snout weakly pointed in lateral view.

Dorsal and anal fins short, extremity slightly pointed in males, rounded in females. Caudal fin oval, slightly longer than deep. Pectoral fin rounded, posterior margin reaching vertical at 80–90% of length between pectoral-fin and pelvic-fin bases. Pelvic fin small, tip reaching between urogenital papilla and base of 1st anal-fin ray in males, reaching between anus and urogenital papilla in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin on vertical through base of 8th anal-fin ray. Dorsal-fin rays 9–11; anal-fin rays 13–15; caudal-fin rays 30–31; pectoral-fin rays 13; pelvic-fin rays 5–6. No contact organs on fins.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 25% of caudal-fin base; no scales on dorsal and anal-fin bases. Frontal squamation F-patterned, rarely E-scale anteriorly overlapping F-scale; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Longitudinal series of scales 29–31;
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**Table 1.** Morphometric data of *Melanorivulus ignescens*.

|                         | holotype | paratypes                      |
|-------------------------|----------|-------------------------------|
|                         |          | male                          | males (n = 9) | females (n = 6) |
| Standard length (mm)    |          | 27.7                          | 20.1–25.5     | 21.4–24.7       |
| Percent of standard length |        |                               |               |
| Body depth              |          | 22.1                          | 21.3–22.3     | 20.5–22.8       |
| Caudal peduncle depth   |          | 13.7                          | 12.9–14.2     | 12.6–13.4       |
| Pre-dorsal length       |          | 77.1                          | 74.0–78.5     | 74.7–77.0       |
| Pre-pelvic length       |          | 55.9                          | 55.7–57.9     | 55.8–57.8       |
| Length of dorsal-fin base |       | 13.5                          | 11.2–12.7     | 10.9–12.8       |
| Length of anal-fin base |          | 23.8                          | 19.8–21.6     | 19.4–21.0       |
| Caudal-fin length       |          | 33.1                          | 29.9–32.9     | 26.8–32.0       |
| Pectoral-fin length     |          | 21.4                          | 19.1–21.5     | 18.0–20.6       |
| Pelvic-fin length       |          | 10.8                          | 9.4–11.1      | 8.2–9.7         |
| Head length             |          | 27.6                          | 27.2–30.9     | 27.2–30.0       |
| Percent of head length  |          |                               |               |
| Head depth              |          | 67.5                          | 62.0–70.4     | 63.3–70.9       |
| Head width              |          | 70.9                          | 66.2–73.3     | 69.6–76.8       |
| Snout length            |          | 13.2                          | 10.9–13.5     | 12.1–14.1       |
| Lower jaw length        |          | 21.6                          | 15.9–19.5     | 15.3–21.4       |
| Eye diameter            |          | 32.3                          | 32.4–35.9     | 32.5–34.7       |

Figure 2. *Melanorivulus ignescens* sp. n., paratype, UFRJ 6876, female, 23.4 mm SL. Photograph by W.J.E.M. Costa.

Transverse series of scales 9; scale rows around caudal peduncle 16. No contact organs on scales. Cephalic neuromasts: supraorbital 3 + 3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1 + 11 + 1, preorbital 2, otic 1, post-otic 1–2, supratemporal 1, median opercular 1, ventral opercular 1, pre-opercular 2 + 4, mandibular 2–3 + 1, lateral mandibular 1, paramandibular 1.
Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight. Ventral process angulo-articular short, pointed. Ventral process of palatine short, slightly contacting quadrate. Mesopterygoid slender, posterior tip not reaching metapterygoid. Metapterygoid sub-rectangular, with constriction on middle portion. Dorsal portion of preopercle short and pointed, channel rudimentary. Basihyal sub-triangular, greatest width 50% of length; basihyal cartilage nearly 15% of total basihyal length. Six branchiostegal rays. Second pharyngobranchial teeth absent. Interarcual cartilage rudimentary. Fourth ceratobranchial teeth present, continuously arranged. Gill-rakers on first branchial arch 1 + 8. Vomerine teeth 2–4. Dermosphenotic present. Ventral process of posttemporal absent. Second proximal radial of dorsal fin between neural spines of 19th and 21st vertebrae, first proximal radial of anal fin between pleural ribs of 13th and 15th vertebrae. Total vertebrae 30–31.

**Colouration. Males.** Flank metallic green-blue to metallic light green, sometimes purple-blue above anal fin; oblique narrow orangish red bars irregularly arranged, often forming chevron-like marks anteriorly directed; horizontal rows of reddish orange dots on anteroventral part of flank, between bases of pectoral and pelvic fins; pale dark grey blotches on postorbital region mainly visible when fish is exposed to strong light. Dorsum light brown with black dots, venter white. Dorsal portion of head side light

**Figure 3.** Geographical distribution of killifishes of the *Melanorivulus dapazi* species group. Yellow circle: *M. ignescens*; black pentagon: *M. flavipinnis*; blue square: *M. regularis*; red star: *M. dapazi*. Blue river drainage: Paraguai; white river drainage: Araguaia.
brown, ventral portion white; pale golden iridescence on opercular region. Jaws dark grey. Iris pale yellow, sometimes with dark brown bar on anterior and posterior portions. Dorsal fin light yellow with two or three oblique dark red bars on posterior portion of fin. Anal fin reddish orange in adult exemplars to yellowish orange in juveniles, basal portion bluish white, distal region becoming gradually dark red-brown, distal margin with high concentration of melanophores. Caudal fin light yellow, often with faint orange spots on middle portion; sometimes pale bluish posterior margin. Pectoral fin hyaline. Pelvic fin orange.

**Females.** Side of trunk and head similar to males, but with paler colours. Ventral surface of head white, with dark grey spots often forming short stripe on chin. Dorsal fin pale yellow, with transverse series of grey spots; broad dark grey to black band on distal margin. Anal fin green-yellow, basal portion light blue with small red spots. Caudal fin pale yellow, with three or four dark grey bars, often interrupted; small black spot, smaller than pupil, on dorso-basal portion of fin overlapping anterior-most bar, more conspicuous in preserved specimens; broad dark grey to black band on whole fin margin.

**Distribution.** Known only from the type locality area, a small stream tributary to the Rio Bandeira, Rio das Garças drainage, upper Rio Araguaia basin, central Brazil, altitude approximately 520 m asl (Fig. 3).

**Etymology.** From the Latin, *ignescens* (becoming inflamed), an allusion to the orange anal fin in males.

*Melanorivulus flavipinnis* sp. n.
[http://zoobank.org/6A6F3FA1-5867-4293-BCC6-D1782B35566C](http://zoobank.org/6A6F3FA1-5867-4293-BCC6-D1782B35566C)
Figs 4–5, Table 2

**Holotype.** UFRJ 6881, male, 28.5 mm SL; Brazil: Mato Grosso state: Rondonópolis municipality: stream tributary of Rio Anhumas, Rio São Lourenço drainage, Rio Paraguai basin, 16°48'16"S, 54°40'52"W, altitude approximately 420 m asl, road BR-070; W. J. E. M. Costa et al., 13 August 2016.

**Paratypes.** UFRJ 6882, 2 males, 25.0–26.6 mm SL, 5 females, 22.3–39.4 mm SL; UFRJ 6883, 2 males, 22.5–25.6 mm SL, 2 females, 24.2–26.4 mm SL (C&S); CICCAA00279, 1 male, 25.7 mm SL, 1 female, 25.3 mm SL; collected with holotype.

**Diagnosis.** *Melanorivulus flavipinnis* differs from all other species of the *M. dapazi* group by the presence, in males, of seven or eight narrow red bars on the caudal fin, irregularly shaped and sometimes interconnected (vs. five or six dark red-brown regularly shaped and never interconnected bars in *M. regularis*; four or fewer short rudimentary bars, sometimes absent, in *M. dapazi*; bars always absent in *M. ignescens*) and by the caudal fin, in females, being yellow on the middle portion and reddish orange on marginal region (vs. yellow to pale pink on the whole fin in the remaining species). Also distinguished from all other congeners of the *M. dapazi* group by the following combination of character states: 5–6 pelvic-fin rays (vs. 7 in *M. dapazi* and *M. regularis*); 30–32 scales in longitudinal series (vs. 35–37 in *M. regularis*); female caudal spot
inconspicuous in live fish (vs. conspicuous in *M. dapazi* and *M. regularis*); caudal fin, in males, without distinctive orange margin (vs. with broad bright orange band along the whole margin in *M. dapazi*); anal fin, in males, yellow (vs. reddish orange in *M. ignescens*); in females, ventral surface of head with dark grey spots, often forming short stripe on chin (vs. without dark grey spots in *M. dapazi*); caudal-fin long, its length 34.1–38.7% SL (vs. short, length 26.8–33.1% SL in *M. ignescens*). Also distinguished from all other species of the *M. dapazi* by the fourth ceratobranchial teeth arranged in two separate sections along the bone surface (vs. continuously arranged).

**Description.** Morphometric data appear in Table 2. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth at vertical just in front of pelvic-fin base. Dorsal and ventral profiles of trunk almost straight to slightly convex in lateral view; dorsal and ventral profiles of caudal peduncle nearly straight. Head moderately wide, sub-triangular in lateral view, dorsal profile nearly straight, ventral profile convex. Jaws short, snout weakly pointed in lateral view. Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight.

Dorsal and anal fins short, tip slightly pointed in males, rounded in females. Caudal fin oval, longer than deep. Pectoral fin rounded, posterior margin reaching vertical at approximately 80–90% of length between pectoral-fin and pelvic-fin bases. Pelvic fin small, tip reaching between base of first and third anal-fin rays in males, reaching urogenital papilla in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin on vertical through base of 8th anal-fin ray. Dorsal-fin rays 9–10; anal-fin rays 14–15; caudal-fin rays 30–31; pectoral-fin rays 13; pelvic-fin rays 5–6. No contact organs on fins.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 25% of caudal-fin base; no scales
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Table 2. Morphometric data of Melanorivulus flavipinnis.

|                      | holotype | males (n = 5) | females (n = 7) |
|----------------------|----------|--------------|-----------------|
| **Standard length (mm)** |          |              |                 |
| Male                  | 28.5     | 22.5–26.6    | 22.9–28.4       |
| **Percent of standard length** |          |              |                 |
| Body depth            | 21.3     | 21.7–22.7    | 20.9–23.1       |
| Caudal peduncle depth | 13.3     | 13.2–14.3    | 12.8–14.1       |
| Pre-dorsal length     | 72.3     | 73.0–76.2    | 73.8–76.4       |
| Pre-pelvic length     | 53.7     | 52.6–55.1    | 53.0–56.8       |
| Length of dorsal-fin base | 14.6 | 11.2–13.7    | 10.4–13.4       |
| Length of anal-fin base | 24.7 | 21.4–25.2    | 20.6–23.3       |
| Caudal-fin length     | 36.1     | 34.1–38.2    | 34.4–38.7       |
| Pectoral-fin length   | 20.7     | 19.9–23.3    | 20.3–21.7       |
| Pelvic-fin length     | 12.4     | 10.6–13.3    | 8.5–11.7        |
| Head length           | 26.3     | 26.1–28.1    | 26.5–28.1       |
| **Percent of head length** |          |              |                 |
| Head depth            | 67.0     | 65.5–71.3    | 66.0–73.5       |
| Head width            | 69.4     | 67.4–72.7    | 70.9–76.2       |
| Snout length          | 13.4     | 12.9–15.3    | 13.1–15.6       |
| Lower jaw length      | 17.5     | 18.4–20.0    | 17.8–19.5       |
| Eye diameter          | 33.4     | 32.3–35.2    | 28.8–35.8       |

Figure 5. Melanorivulus flavipinnis sp. n., paratype, UFRJ 6882, female, 27.8 mm SL. Photograph by W.J.E.M. Costa.

on dorsal and anal-fin bases. Frontal squamation F-patterned, rarely E-scale anteriorly overlapping F-scale; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Longitudinal series of scales 30–32; transverse series of scales 8; scale rows around caudal peduncle 16. No contact organs on scales. Cephalic neuromasts: supraorbital 3 + 3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1 + 10–11 + 1, preorbital 2, otic 1, post-otic 1, supratemporal 1,
median opercular 1, ventral opercular 1, pre-opercular 2 + 4, mandibular 2–3 + 1, lateral mandibular 1, paramandibular 1.

Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight. Ventral process angulo-articular short, pointed. Ventral process of palatine short, slightly contacting quadrate. Mesopterygoid slender, posterior tip not reaching metapterygoid. Metapterygoid sub-rectangular, with constriction on middle portion. Dorsal portion of preopercle short and pointed, channel rudimentary. Basihyal sub-triangular, greatest width about 50% of length; basihyal cartilage about 15–25% of total basihyal length. Six branchiostegal rays. Second pharyngobranchial teeth absent. Interarcual cartilage rudimentary. Fourth ceratobranchial teeth present, continuously arranged. Gill-rakers on first branchial arch 1 + 8. Vomerine teeth 2. Dermosphenotic present. Ventral process of posttemporal absent. Second proximal radial of dorsal fin between neural spines of 19th and 21st vertebrae, first proximal radial of anal fin between pleural ribs of 13th and 15th vertebrae. Total vertebrae 30–31.

**Colouration. Males.** Flank metallic green-blue to metallic light blue, sometimes purple-blue above anal fin; oblique narrow orangish red bars irregularly arranged, often forming chevron-like marks anteriorly directed; short light red stripe on humeral region; horizontal rows of reddish orange dots on antero-ventral part of flank, between bases of pectoral and pelvic fins; pale dark grey blotches on postorbital region mainly visible when fish is exposed to strong light. Dorsum light yellowish-brown with black dots, venter white. Dorsal portion of head side light brown, ventral portion white; pale golden iridescence on opercular region. Jaws dark grey. Iris pale yellow, sometimes with dark brown bar on anterior and posterior portions. Dorsal fin light yellow with seven or eight narrow oblique red bars, often forming reticulate pattern on distal portion of fin. Anal fin pale blue on its proximal half, with faint oblique red bars, light yellow in its distal half, distal region becoming gradually dark reddish brown on marginal border, distal margin with high concentration of melanophores. Caudal fin bright yellow, more intensely pigmented on dorsal and ventral portions, with seven or eight narrow red bars, irregularly shaped and sometimes interconnected. Pectoral fin yellowish hyaline. Pelvic fin light blue with orangish brown anterior margin.

**Females.** Side of trunk and head similar to males, but with paler colours. Ventral surface of head white, with dark grey spots often forming short stripe on chin. Dorsal fin pale yellow, with oblique grey bars; broad dark grey to black band on distal margin. Anal fin green-yellow, basal portion light blue with small red spots. Caudal fin pale yellow on middle portion, reddish orange on marginal region, with five to seven dark grey bars, often interconnected; small black spot, smaller than pupil, on dorso-basal portion of fin overlapping anterior-most bar, conspicuous only in preserved specimens; broad dark grey to black band on whole fin margin.

**Distribution.** Known only from the type locality, a small stream tributary to the Rio Anhumas, Rio São Lourenço drainage, Rio Paraguai basin, central Brazil, altitude approximately 420 m asl (Fig. 3).

**Etymology.** From the Latin, *flavipinnis* (yellow fins), referring to the bright yellow colouration of the caudal fin in males.
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Melanorivulus regularis sp. n.
http://zoobank.org/6DE3B93B-9257-4557-B4C1-20B4D2BD05FE
Fig 6, Table 3

Holotype. UFRJ 6878, male, 26.9 mm SL; Brazil: Mato Grosso state: Alto Graças municipality: Ribeirão da Sobra, upper Rio Itiquira drainage, Rio Paraguai basin, 16°54’41”S, 53°37’55”W, altitude approximately 750 m asl, road BR-364; W. J. E. M. Costa et al., 5 August 2016.

Paratypes. UFRJ 6879, 4 males, 24.4–33.3 mm SL, 9 females, 22.3–33.8 mm SL; UFRJ 6880, 2 males, 25.3–31.2 mm SL, 2 females, 23.7–28.4 mm SL (C&S); CICCAA00278, 1 male, 24.7 mm SL, 1 female, 25.9 mm SL; collected with holotype.

Diagnosis. Melanorivulus regularis is distinguished from all other species of the M. dapazi group by the presence, in males, of five or six dark reddish brown, regularly shaped and never interconnected bars on the caudal fin (vs. seven or eight narrow red bars, irregularly shaped and sometimes interconnected in M. flavipinnis; four or fewer short rudimentary bars, sometimes absent, in M. dapazi; bars always absent in M. ignescens). Also distinguished from all other congeners of the M. dapazi group by the following combination of character states: 7 pelvic-fin rays (vs. 5–6 in M. flavipinnis and M. ignescens); 35–37 scales in longitudinal series (vs. 29–32 in M. flavipinnis and M. ignescens); caudal fin, in females, pale (vs. yellow on the middle portion and reddish orange on marginal region in M. flavipinnis); female caudal spot conspicuous in live exemplars fish (vs. inconspicuous in M. flavipinnis and M. ignescens); caudal fin, in males, without distinctive orange margin (vs. with broad bright orange band along the whole margin in M. dapazi); anal fin, in males, yellow (vs. reddish orange in M. ignescens); in females, ventral surface of head with dark grey spots, often forming short stripe on chin (vs. without dark grey spots in M. dapazi). Also distinguished from all other congeners of the M. dapazi group by having 32 vertebrae (vs. 29–31)

Description. Morphometric data appear in Table 3. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth at vertical just in front of pelvic-fin base. Dorsal and ventral profiles of trunk almost straight to slightly convex in lateral view; dorsal and ventral profiles of caudal peduncle nearly straight. Head moderately wide, sub-triangular in lateral view, dorsal profile nearly straight, ventral profile convex. Jaws short, snout weakly pointed in lateral view. Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight.

Dorsal and anal fins short, tip slightly pointed in males, rounded in females. Caudal fin oval, slightly longer than deep. Pectoral fin rounded, posterior margin reaching vertical at around 80% of length between pectoral-fin and pelvic-fin bases. Pelvic fin small, tip reaching between urogenital papilla and base of 1st anal-fin ray in males, reaching anus in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin on vertical through base of 8th anal-fin ray. Dorsal-fin rays 10–11; anal-fin rays 14–15; caudal-fin rays 31–33; pectoral-fin rays 13–14; pelvic-fin rays 7. No contact organs on fins.
Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 25% of caudal-fin base; no scales on dorsal and anal-fin bases. Frontal squamation F-patterned, rarely E-scale anteriorly overlapping F-scale; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Longitudinal series of scales 35–37; transverse series of scales 9; scale rows around caudal peduncle 16. No contact organs on scales. Cephalic neuromasts: supraorbital 3 + 3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1 + 10–11 + 1, preorbital 1–2, otic 1, post-otic 1, supratemporal 1, median opercular 1, ventral opercular 1, pre-opercular 2 + 4, mandibular 2–3 + 1, lateral mandibular 1, paramandibular 1.

Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight. Ventral process angulo-articular short, pointed. Ventral process of palatine short, slightly contacting quadract. Mesopterygoid slender, posterior tip not reaching metapterygoid. Metapterygoid sub-rectangular, with constriction on middle portion. Dorsal portion of preopercle short and pointed, channel rudimentary. Basihyal sub-triangular, greatest width near 55% of length; basihyal cartilage the 20% of total basihyal length. Six branchiostegal rays. Second pharyngobranchial teeth absent. Interarcual cartilage rudimentary. Fourth ceratobranchial teeth present, continuously arranged. Gill-rakers on first branchial arch 1 + 7–8. Vomerine teeth 2–5. Dermosphenotic present. Ventral process of posttemporal absent. Second proximal radial of dorsal fin between neural spines of 19th and 21st vertebrae, first proximal radial of anal fin between pleural ribs of 14th and 15th vertebrae. Total vertebrae 32.

**Colouration. Males.** Flank light metallic blue; oblique narrow orange-red bars irregularly arranged, often forming chevron-like marks anteriorly directed; horizontal rows of reddish orange dots on antero-ventral part of flank, between bases of pectoral and pelvic fins; dark brown pigmentation concentrated on postorbital, overlapped by black dots on superficial layer of skin. Dorsum light yellowish-grey, venter white. Dor-
Three new species of the killifish genus *Melanorivulus* from the central Brazilian Cerrado...

### Table 3. Morphometric data of *Melanorivulus regularis*.

|                             | holotype | paratypes                  |
|-----------------------------|----------|----------------------------|
|                             | male     | males (n = 7)               |
| Standard length (mm)        | 26.9     | 24.4–33.3                  |
| Percent of standard length  |          |                            |
| Body depth                  | 23.2     | 21.9–22.6                  |
| Caudal peduncle depth       | 13.4     | 12.7–13.9                  |
| Pre-dorsal length           | 73.9     | 72.7–76.5                  |
| Pre-pelvic length           | 55.4     | 53.8–58.0                  |
| Length of dorsal-fin base   | 13.6     | 11.5–13.9                  |
| Length of anal-fin base     | 22.1     | 20.4–24.1                  |
| Caudal-fin length           | 34.1     | 32.7–33.7                  |
| Pectoral-fin length         | 21.4     | 20.0–22.2                  |
| Pelvic-fin length           | 10.7     | 9.6–12.4                   |
| Head length                 | 27.2     | 25.3–27.5                  |

| Percent of head length       |          |                            |
| Head depth                  | 69.6     | 67.9–73.1                  |
| Head width                  | 73.5     | 71.1–77.2                  |
| Snout length                | 15.2     | 13.6–17.1                  |
| Lower jaw length            | 20.1     | 16.6–20.7                  |
| Eye diameter                | 29.3     | 30.1–33.5                  |

### Description

**Males.** Sal portion of head side light brown, ventral portion white; pale golden iridescence on opercular region. Jaws dark grey. Iris pale yellow to pale brown. Dorsal fin pale yellow with four or five narrow red bars on posterior portion of fin. Anal fin orangish-yellow, basal portion white, posterior portion pale blue with two or three faint red oblique bars; distal region becoming gradually dark red-brown, distal margin with high concentration of melanophores. Caudal fin pale blue to pale yellow, with five or six dark red-brown regularly shaped bars, ventral portion light yellow without bars, ventral margin orangish-brown. Pectoral fin yellowish-hyaline. Pelvic fin pale blue with brown anterior margin.

**Females.** Side of trunk and head similar to males, but with paler colours. Ventral surface of head white, with dark grey spots often forming short stripe on chin. Dorsal fin pale yellow, with three or four bars on posterior region; broad dark grey to black band on distal margin. Anal fin pale yellow, basal portion light blue. Caudal fin pale yellow, with four or five dark grey bars; small black spot, slightly smaller than pupil, on dorso-basal portion of fin; broad dark grey to black band on whole fin margin.

**Distribution.** Known only from the type locality, Ribeirão da Sobra, an upper tributary of the Rio Itiquira, Rio Paraguai basin, central Brazil, in altitude about 750 m asl (Fig. 3).

**Etymology.** From the Latin, *regularis* (regular), a reference to the caudal fin bars in males, regularly shaped and arranged on fin.
Key to the species of the Melanorivulus dapazi group

1  In females, ventral surface of head with dark grey spots, often forming short stripe on chin; in males, caudal fin never with broad bright orange band along entire margin ........................................................................................................2

   – In females, ventral surface of head without dark grey spots; in males, caudal fin with broad bright orange band along margin .......... M. dapazi

2  7 pelvic-fin rays; 35–37 scales in longitudinal series; female caudal spot conspicuous in live fish ................................................................. M. regularis

   – 5–6 pelvic-fin rays; 29–32 scales in longitudinal series; female caudal spot inconspicuous in live fish .......................................................... M. flavipinnis

   4 Caudal fin, in males, without bars; caudal fin, in females, pale yellow; anal fin, in males, bright red-orange; caudal-fin length 26.8–33.1% SL ...... M. ignescens

   – Caudal fin, in males, with 7–8 red bars; caudal fin, in females, pale yellow on middle portion and orange on marginal portion; anal fin, in males, light yellow; caudal-fin length 34.1–38.7% SL .......................... M. flavipinnis

Discussion

Morphological characters indicate that all three new species here described are more closely related to M. dapazi than to other congeners, with these four species comprising the M. dapazi group. In all species of this group, the interarcual cartilage is rudimentary, nearly equal in size to the adjacent cartilage at the tip of the first epibranchial (Fig. 7). In other species of Melanorivulus, the cartilage is well-developed, larger than first epibranchial cartilage, and around one fourth the length of the first epibranchial (e.g., Costa 2016: fig. 4). In addition, species of the M. dapazi group share the presence of a dark red-brown distal margin on the male anal fin (Figs 1, 4, 6), a condition not found in other congeners. A molecular phylogeny of Melanorivulus (Costa et al. 2016) supports M. dapazi as a sister group to a clade including species of the M. decoratus group, but the three species here described were not included in that analysis. The M. decoratus species group is diagnosed by the presence of five branchiostegal rays (vs. six) and a narrow basihyal, its width around 35% of the longitudinal length (vs. 45–60%). The M. decoratus species group is comprised of three miniature species not surpassing 20 mm SL: M. atlanticus Costa, Bragança & Ottoni, 2015, from the coastal plains of northeastern Brazil, M. decoratus Costa, 1989, from the middle Rio São Francisco Basin, and M. jalapensis Costa, 2010 from the middle Rio Tocantins drainage (Costa 1989, 2010; Costa et al. 2015).

Relationships among species of the M. dapazi group remain unclear. Melanorivulus flavipinnis, endemic to the Paraguai basin, is possibly more closely related to M. ignescens, endemic to the Araguaia basin, than to M. dapazi and M. regularis that like M. flavipinnis are endemic to the Paraguai basin. Among species of the M. dapazi group, only in M. flavipinnis and M. ignescens there are five or six rays in the pelvic
fin. In addition, in both species the spot on the basal portion of the female caudal fin is inconspicuous in live fish (Figs 2 and 5) and poorly visible in preserved specimens. In *M. dapazi* and *M. regularis*, there are seven pelvic-fin rays and the female caudal spot is conspicuous and delimited in live (Fig. 8) and preserved specimens, conditions considered plesiomorphic for *Melanorivulus* (Costa, 2016). The unique pigmentation pattern on the ventral surface of the head in females that is shared by *M. flavipinnis*,

Figure 7. Dorsal branchial arches, left side, ventral view, of *Melanorivulus flavipinnis*, paratype, UFRJ 6883, 25.6 mm SL. Larger stippling indicates cartilage. Scale bar: 0.5 mm.

Figure 8. *Melanorivulus dapazi*, UFRJ 11203, female, 22.2 mm SL. Photograph by W.J.E.M. Costa.
M. ignescens, and M. regularis (Fig. 9), may be indicative of close relationships among these three species.

Costa (2016) discussed the importance of using live colour pattern characters to diagnose species and species groups of Melanorivulus, showing high congruence with molecular data. In that study, particular attention was given to patterns involving the caudal fin, which contained a high concentration of phylogenetically informative characters, useful to delimit most species of the M. zygonectes group. Concordantly, the present study shows that colour patterns documented from live fish is an accurate tool to recognise species of the M. dapazi group (see key for species identification above).
Osteological characters have been used to infer relationships among species groups of *Melanorivulus* and for diagnostic purposes (e.g., Costa 2016; this study). The present study shows that osteological characters may be also useful to diagnose single species. The unique shape of the metapterygoid recorded for *M. ignescens*, with a constriction in its middle portion (Fig. 10), and the unique arrangement of teeth on the fourth ceratobranchial in *M. flavipinnis*, exhibiting a median gap (Fig. 11), are not present in other congeners. In addition, *M. regularis* differs from other species of the *M. dapazi* group by having 32 vertebrae (vs. 29–31 in other species). Thus, although checking osteological characters in larger specimen samples is often not practicable, it is recommendable that osteology be included in taxonomical studies of *Melanorivulus* to complement species diagnoses.

**Figure 11.** Basihyal and ventral branchial arches, right and median portion, dorsal view, of *Melanorivulus flavipinnis*, paratype, UFRJ 6883, 25.6 mm SL. Larger stippling indicates cartilage. Scale bar: 0.5 mm.
Recent killifish inventories in the area of the central Brazilian plateaus drained by the upper tributaries of the Araguaia, Paraná and Paraguai river basins have revealed an unexpected high diversity of species of the genus *Melanorivulus* (e.g., Costa 2012). The present study confirms this high diversity, indicating once again that species inhabiting the region have small geographical ranges, often restricted to short segments of a single river drainage. For example, among species endemic to the Paraguai basin, *M. regularis* was found in a single locality of the Rio Itiquira drainage, at approximately 750 m asl, whereas the present field survey indicated that at altitudes around 450 m asl of the same drainage, the only species found was *M. dapazi*, which also occurs in similar altitudes of the neighbouring areas included in the Rio Correntes drainage (Costa 2005). On the other hand, *M. flavipinnis* here described from the Rio São Lourenço drainage at approximately 420 m asl, is substituted by *M. cyanopterus* (Costa, 2005), at altitudes of approximately 250 m asl (Costa 2005). The last species is a member of the *M. punctatus* group, distantly related to the *M. dapazi* group and geographically widespread in the lower Paraguai river basin (Costa et al. 2016). Recent studies with other vertebrates occurring in the Cerrado indicate that high species diversity in the region is correlated with the topographical reorganization during the Miocene, which generated geographical isolation of ancestral populations in plateaus and peripheral depressions (Prado et al. 2012; Guarnizo et al. 2016). This paleogeographical scenario may explain the present distribution of distinct species of *Melanorivulus* along different altitudinal zones of river drainages.

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**References**

Costa WJEM (1988) Sistemática e distribuição do complexo de espécies *Cynolebias minimus* (Cyprinodontiformes, Rivulidae), com a descrição de duas espécies novas. Revista Brasileira de Zoologia 5: 557–570. https://doi.org/10.1590/S0101-81751988000400004

Costa WJEM (1989) Descrição de cinco novas espécies de *Rivulus* das bacias dos rios Paraná e São Francisco (Cyprinodontiformes, Rivulidae). Revista Brasileira de Zoologia 6: 523–534. https://doi.org/10.1590/S0101-8175198900300012

Costa WJEM (2001) The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. Ichthyological Exploration of Freshwaters 12: 333–383.
Three new species of the killifish genus Melanorivulus from the central Brazilian Cerrado...

Costa WJEM (2005) Seven new species of the killifish genus Rivulus (Cyprinodontiformes: Rivulidae) from the Paraná, Paraguay and upper Araguaia river basins, central Brazil. Neotropical Ichthyology 3: 69–82. http://dx.doi.org/10.1590/S1679-62252005000100003

Costa WJEM (2006a) Rivulus kayapo n. sp. (Teleostei: Cyprinodontiformes: Rivulidae): a new killifish from the serra dos Caiapós, upper rio Araguaia basin, Brazil. Zootaxa 1368: 49–56. http://www.mapress.com/zootaxa/2006f/z01368p056f.pdf

Costa WJEM (2006b) Descriptive morphology and phylogenetic relationships among species of the Neotropical annual killifish genera Nematolebias and Simpsonichthys (Cyprinodontiformes: Aplocheiloidei: Rivulidae). Neotropical Ichthyology 4: 1–26. http://dx.doi.org/10.1590/S1679-62252005000100001

Costa WJEM (2007a) Rivulus illuminatus, a new killifish from the serra dos Caiapós, upper rio Paraná basin, Brazil (Teleostei: Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 18: 193–198.

Costa WJEM (2007b) A new species of Rivulus from the Claro river drainage, upper Paraná river basin, central Brazil, with redescription of R. pinima and R. vittatus (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 18: 313–323.

Costa WJEM (2008) Rivulus formosensis, a new aplocheiloid killifish from the upper Corrente River drainage, upper Paraná River basin, central Brazil. Ichthyological Exploration of Freshwaters 19: 85–90.

Costa WJEM (2010) Rivulus jalapensis, a new killifish from the Tocantins River basin, central Brazil (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 21: 193–198.

Costa WJEM (2012) Two new species of Melanorivulus from the Caiapós hill, upper Araguaia river basin, Brazil (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 23: 211–218.

Costa WJEM (2016) Comparative morphology, phylogenetic relationships, and taxonomic revision of South American killifishes of the Melanorivulus zygonectes species group (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 27: 107–152.

Costa WJEM, Amorim PF, Rizzieri RC (2016) Molecular phylogeny and biogeography of the South American savanna killifish genus Melanorivulus (Teleostei: Rivulidae). Vertebrate Zoology 66: 267–273. http://www.senckenberg.de/files/content/forschung/publikationen/vertebratezoology/vz66-3/04_vertebrate_zoology_66-3_costa_267-273.pdf

Costa WJEM, Bragança PHN, Ortoni FP (2015) A new miniature killifish of the genus Melanorivulus (Cyprinodontiformes: Rivulidae) from the coastal plains of north-eastern Brazil. Vertebrate Zoology 65: 31–35. http://www.senckenberg.de/files/content/forschung/publikationen/vertebratezoology/vz65-1/04_vertebrate_zoology_65-1_costa_et_al_31-35.pdf

Guarnizo CE, Werneck FP, Giugliano LG, Santos MG, Fenker J, Sousa L, D’Angiolella AB, dos Santos AR, Strüssmann C, Rodrigues MT, Dorado-Rodrigues TF, Gamble T, Colli GR (2016) Cryptic lineages and diversification of an endemic anole lizard (Squamata, Dactyloidae) of the Cerrado hotspot Molecular Phylogenetics and Evolution 94: 279–289. http://dx.doi.org/10.1016/j.ympev.2015.09.005

Hoedeman JJ (1958) The frontal scalation pattern in some groups of toothcarts (Pisces, Cyprinodontiformes). Bulletin of Aquatic Biology 1: 23–28.
Myers N, Mittermeir RA, Mittermeir CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501

Oliveira LE, Facure KG, Giaretta AA (2012) Habitat, density, and spatial distribution of Rivulus giarettai (Actinopterygii, Cyprinodontiformes) in southeastern Brazil. Environmental Biology of Fishes 93: 89–94. https://doi.org/10.1007/s10641-011-9892-1

Prado CPA, Haddad CFB, Zamudio KR (2012) Cryptic lineages and Pleistocene population expansion in a Brazilian Cerrado frog. Molecular Ecology 21: 921–941. https://doi.org/10.1111/j.1365-294X.2011.05409.x

Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–109. http://sfi.mnhn.fr/cybium/numeros/1985/92/01-Taylor%5b92%5d107-119.pdf