

Taxonomy of the seasonal killifish genus *Neofundulus* in the Brazilian Pantanal (Cyprinodontiformes: Rivulidae)

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 21944-970, Rio de Janeiro, Brasil; wcosta(at)acd.ufrj.br

Accepted 19.ii.2015.

Published online at www.senckenberg.de/vertebrate-zoology on 4.v.2014.

Abstract

On the basis of fish collections made between 1991 and 2014, four species of the seasonal killifish genus *Neofundulus* are reported to occur in the Brazilian Pantanal, Paraguay river basin: *N. parvipinnis*, endemic to the Cuiabá and São Lourenço river drainages, in the northern portion of the Pantanal; *N. rubrofasciatus*, new species, from the Miranda river drainage, *N. aureomaculatus*, new species, from the Aquidauana river drainage, both in the south-eastern portion of the Pantanal; and *N. paraguayensis*, occurring in the Paraguay and Nabileque river floodplains, in the southern part of the Pantanal. The new species are diagnosed by unique colour patterns, and a combination of morphological characters states indicating that they are more closely related to *N. parvipinnis* and *N. splendidus* than to *N. paraguayensis*.

Key words

Aplocheiloid killifishes, Biodiversity, Pantanal wetland, Paraguay river, Systematics.

Introduction

The Brazilian Pantanal, also known as Pantanal de Mato Grosso, is a vast wetland region, about 140,000 km², comprising a tectonic depression along the left margin of the Paraguay river. It is situated in a savannah transitional zone between the biogeographical provinces of the Cerrado in the north and west, and the Chaco in the south and east, being remarkable by the rich biodiversity containing elements from both provinces. Consisting of a poorly drained lowland area with annual flooding period between December and June, the Pantanal concentrates a great diversity of ephemeral aquatic habitats suitable for the occurrence of seasonal rivulid killifishes, which are there represented by six genera: *Moema* COSTA, 1989, *Neofundulus* MYERS, 1924, *Plesiolebias* COSTA, 1989, *Pterolebias* GARMAN, 1895, *Stenolebias* COSTA, 1995, and *Trigonectes* MYERS (*e.g.*, COSTA, 1988a, 1988b, 1990, 1995a, 2003, 2005, 2007).

Neofundulus has been known for a few papers in the scientific literature. Until 1988, knowledge about *Neofundulus* was restricted to the original descriptions of *N. paraguayensis* (EIGENMANN & KENNEDY, 1903) and *N. ornatipinnis* MYERS, 1935, each based on a single specimen collected in the Paraguayan Chaco (EIGENMANN & KENNEDY, 1903; MYERS, 1935), besides records of a few specimens from near Cuiabá, central Brazil (MYERS, 1942; SANTOS, 1979) and a few specimens from the Argentinean Chaco (ARÁMBURU *et al.*, 1962). COSTA (1988a) tentatively revised the genus on the basis of the few specimens available in collections, recognising two species in the Brazilian Pantanal, *N. parvipinnis* COSTA, 1988 in the northern area, and *N. paraguayensis* in the southern. Subsequent collections in the Pantanal provide the basis for the present taxonomical revision, which also includes first remarks on the distribution and habitat of endemic species.



Fig. 1. *Neofundulus parvipinnis*: UFRJ 4886, male, 33.6 mm SL: Brazil: Mato Grosso: Joselândia.

Material and methods

Material examined is deposited in the following institutions: CAS-SU, California Academy of Sciences, San Francisco, MLP, Museo La Plata, La Plata, MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo, and UFRJ, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro. Morphological characters were obtained from specimens fixed in formalin just after collection, for a period of 10 days, and then transferred to 70 % ethanol. Data on colour patterns were based both on direct examination of live specimens during collections, and numerous photographs of both sides of live individuals, at least two males and one female for each collection, taken in aquaria between 20 and 24 hours after collection. In descriptions, the eight spot rows or stripes on the flank in males are numbered from top to below. Morphometric and meristic data were taken following COSTA (1988c); measurements are presented as percent of standard length (SL), except for those related to head morphology, which are expressed as percent of head length. Fin-ray counts include all elements. Number of vertebrae and gill-rakers were recorded from cleared and stained specimens; the compound caudal centrum was counted as a single element. Osteological preparations (c&s) were made according to TAYLOR & VAN DYKE (1985). Terminology for frontal squamation follows HOEDEMAN (1958) and for cephalic neuromast series COSTA (2001). Species were diagnosed using two criteria: unique character states (autapomorphic criterion; e.g., ROSEN, 1979), followed by a unique combination of character states (diagnosability criterion; e.g., DAVIS & NIXON, 1992). Comparisons did not include *Neofundulus guaporensis* COSTA, 1988, a species known from a single juvenile specimen, having its generic posi-

tion still uncertain, but readily differing from all species of *Neofundulus* by having 12 anal-fin rays (vs. 14–18) (COSTA, 1988a).

Neofundulus parvipinnis COSTA, 1988

Fig. 1

Neofundulus parvipinnis COSTA, 1988: 109 (type locality: lagoon at right margin of the road BR-364, 36 km east of Cuiabá, Mato Grosso, Brazil [Cuiabá river drainage, Paraguay river basin, 15°42'17"S 55°42'40"W]; holotype: MZUSP 36618, male, 53.5 mm SL).

Material examined: All from the Paraguay river basin in Estado de Mato Grosso, central Brazil. Cuiabá river drainage: MZUSP 36618, holotype; MZUSP 36619, paratype; MZUSP 36620, 5 paratypes; Município de Cuiabá: lagoon at right margin of the road BR-364, 36 km east of city of Cuiabá, 15°42'17"S 55°42'40"W; P. S. Santos Filho, 14 Jan. 1976. – MZUSP 36687, 1 paratype; seasonal lagoon near Coxipó river, village of Coxipó da Ponte; K. Silimon, no date. – UFRJ 267, 1 (C&S); near Cuiabá; J.D. SOARES, March 1990. São Lourenço river drainage, Município de Joselândia: UFRJ 4886, 8; UFRJ 10081, 5 (C&S); 5 km NW of Posto São Luis, RPPN SESC-Pantanal, 16°39'57"S 56°08'02"W; W.J.E.M. COSTA *et al.*, 10 June 1999. – UFRJ 5836, 3; near Posto São Luis, RPPN SESC-Pantanal; J. ALVES, 28 May 2003.

Diagnosis. Distinguished from all congeners by having dorsal-fin base smaller and dorsal fin more posteriorly positioned in males (dorsal-fin base length 13.6–16.9 % SL, vs. 17.5–21.3 % SL; pre-dorsal length 71.3–74.4 % SL, vs. 63.6–69.3 % SL) and head more slender in males (head depth 70.5–73.4 % of head length vs. 74.7–84.8 % head length). Also distinguished from all species, except *N. aureomaculatus*, *N. rubrofasciatus* and

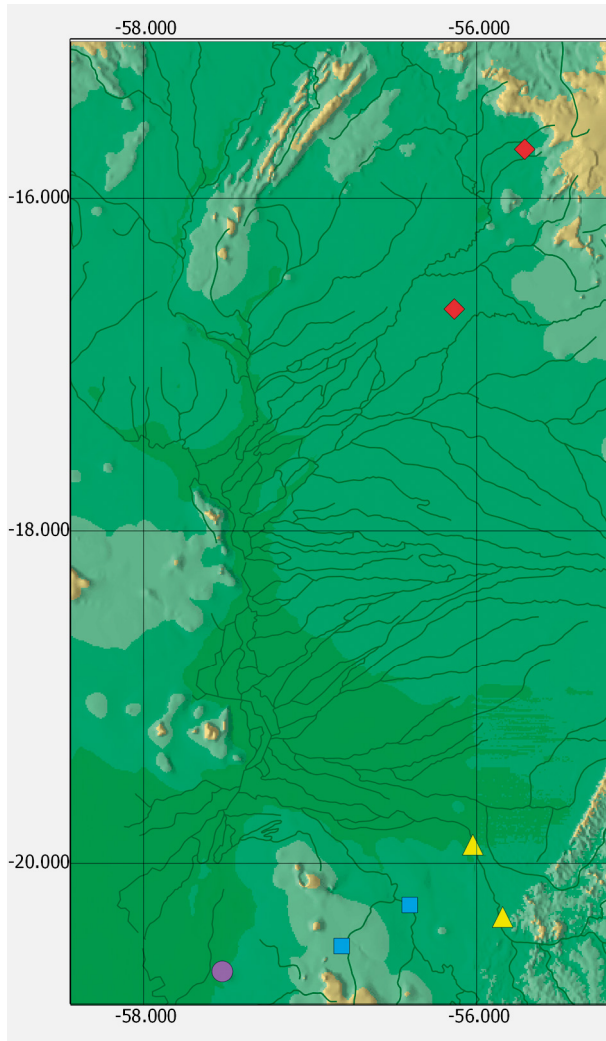


Fig. 2. Geographical distribution of the genus *Neofundulus* in the Brazilian Pantanal: red lozenge, *N. parvipinnis*; yellow triangle, *N. aureomaculatus*; blue square, *N. rubrofasciatus*; purple dot, *N. paraguayensis*.

N. splendidus, by the presence of highly contrasting red or purple stripes and golden stripes on the caudal peduncle in males (vs. horizontal rows of dark reddish brown and pale yellow spots), and rows of golden spots placed between flank stripes on the anterior-most part of the flank in males (vs. golden spots absent); from *N. aureomaculatus*, *N. rubrofasciatus*, and *N. splendidus*, by having a small rounded black or dark grey humeral spot in males, smaller than orbit, often with vertical extensions (vs. large round spot, larger than orbit); from *N. rubrofasciatus*, by the absence of bright yellow zigzag horizontal lines on the anterior-most portion of the flank in males (vs. presence), basal portion of anal fin dark purple in males (vs. bright red), and presence of vertical series of golden spots on the anterior portion of the flank (vs. absence); from *N. splendidus* by having 34–36 scales on the longitudinal series (vs. 40–41) and absence of black bars on the dorsal fin in males (vs. presence); from *N. aureomaculatus* by the absence of golden spots

distinctively larger than red spots on the anterior portion of flank rows 3 and 4 (vs. presence) and shorter jaw in males (lower jaw length 17.4–19.4 % of head length vs. 20.8–24.0 %); and from *N. paraguayensis* by having 16 rows of scales around caudal peduncle (vs. 20), fewer dorsal-fin rays (11–13 vs. 14–16), 1–2+8 gill-rakers on the first branchial arch (vs. 3+10–11), a narrower basi-hyal (width about 65–70 % of basi-hyal length, vs. about 75–95 %), and dorsal and ventral hypurals plates separated (vs. united).

Distribution and habitat. *Neofundulus parvipinnis* is endemic to the northern Pantanal, occurring in the Cuiabá and São Lourenço river drainages (Fig. 2). Like other congeners occurring in the Pantanal, it inhabits shallow temporary pools, about 30–50 cm deep, where other rivulids are not found. In the São Lourenço river drainage, its habitat is well-preserved by being situated into a private environmental protected area (Reserva Particular do Patrimônio Natural SESC Pantanal). Conservation of the habitats in the area of the type locality in the Cuiabá river drainage has not been assessed in recent years.

Neofundulus rubrofasciatus, new species

Fig. 3, Table 1

Holotype: UFRJ 10083, male, 43.3 mm SL; Brazil: Estado de Mato Grosso do Sul: Município de Bodoquena: temporary pool about 18 km W of the village of Bodoquena, road MS-339, Salobra river floodplains, Miranda river drainage, Paraguay river basin, 20°29'42"S 56°48'37"W, altitude about 170 m; W.J.E.M. COSTA *et al.*, 22 July 2014.

Paratypes: UFRJ 10084, 1 male, 32.4 mm SL, 12 females, 26.5–33.8 mm SL; UFRJ 10085, 1 male, 33.6 mm SL, 4 females, 27.4–29.4 mm SL (C&S); UFRJ 10086, 4 females, 23.1–26.0 mm SL; collected with holotype.

Additional material (non-types): UFRJ 1012, 2; Brazil: Estado de Mato Grosso do Sul: Município de Miranda: temporary pool near the town of Miranda, in the road to Bodoquena, Miranda river floodplains, Paraguay river basin, 20°14'56"S 56°24'06"W, about 115 m of altitude; W.J.E.M. COSTA *et al.*, 18 March 1991.

Diagnosis. Distinguished from all congeners by two characters of the colour pattern, comprising the presence of bright yellow zigzag horizontal lines on the anterior portion of the flank in males, between flank stripes (vs. short straight rows of yellow spots, when yellow marks present in this part of the flank), and basal portion of anal fin red in males (vs. purple). Also distinguished from all species, except *N. aureomaculatus*, *N. parvipinnis* and *N. splendidus*, by the presence of highly contrasting red or purple stripes and golden stripes on the caudal peduncle in males (vs. horizontal rows of dark reddish brown and pale yellow spots), and rows of golden spots



Fig. 3. *Neofundulus rubrofasciatus*: A, UFRJ 10083, holotype, male, 43.3 mm SL; B, UFRJ 10084, paratype, male, 32.4 mm SL; C, UFRJ 10084, paratype, female, 33.8 mm SL: Brazil: Mato Grosso do Sul: Bodoquena.

placed between flank stripes on the anterior-most part of the flank in males (vs. golden spots absent); from *N. aureomaculatus*, *N. parvipinnis* and *N. splendidus* by the absence of vertical series of golden spots on the anterior portion of the flank (vs. presence); from all congeners, except *N. aureomaculatus* and *N. splendidus*, by having a round black or dark grey humeral spot, larger than orbit

at least in males (vs. vertically elongated, its horizontal width smaller than orbit); from *N. splendidus* by having 35–37 scales on the longitudinal series (vs. 40–41), 14 or 15 dorsal-fin rays (vs. 10–13), and absence of black bars on the dorsal fin in males (vs. presence); from *N. aureomaculatus* by the absence of golden spots distinctively larger than red spots on the anterior portion of flank rows

3 and 4 (vs. presence), scales extending on anterior 30 % of the caudal in both sexes (vs. about 45–50 % in males), and shorter jaw in males (lower jaw length 16.1–20.6 % of head length vs. 20.8–24.0 %); from *N. parvipinnis* by having flank stripes narrower than interspace in males (vs. flank stripes occupying almost entire interspace), dorsal-fin base larger and dorsal fin slightly more anteriorly positioned in males (dorsal-fin base length 18.3–20.8 % SL, vs. 13.7–16.9 % SL; pre-dorsal length 67.6–69.3 % SL, vs. 71.3–73.7 % SL; and dorsal-fin origin between neural spines of vertebrae 18 and 19 vs. 20 and 21) and head deeper and wider (head depth 74.7–80.0 % of head length in males and 77.2–79.7 % in females, head width 77.9–83.5 % of head length in males and 80.8–85.4 % in females, vs. 70.5–73.4 % of head length in males and 68.4–75.4 % in females, head width 67.4–76.2 % of head length in males and 73.1–77.2 % in females); and from *N. paraguayensis* by having 16 rows of scales around caudal peduncle (vs. 20), 1–2+8 gill-rakers on the first branchial arch (vs. 3+10–11), a narrower basihyal (width about 65–70 % of basihyal length, vs. about 75–95 %), and dorsal and ventral hypurals plates separated (vs. united).

Description. Morphometric data appear in Table 1. Largest male examined 46.3 mm SL; largest female examined 33.8 mm SL. Dorsal profile slightly convex and ventral profile approximately straight between snout and anterior part of caudal peduncle, nearly straight to caudal-fin base. Body slender, greatest body depth in vertical just anterior to pelvic-fin insertion. Urogenital papilla small, globular in males, urogenital opening placed in pocket-like structure in females. Longitudinal series of scales 35–37; transverse series of scales 9–10; scale rows around caudal peduncle 16. One neuromast on each scale of lateral line. No contact organ on scales. Total vertebrae 34–35.

Eye positioned on dorsal portion of head side. Snout short, blunt. Premaxilla and dentary teeth conical, small, numerous, irregularly arranged, except for external series with longer fang-like teeth, more robust and more curved inside mouth in males. Vomerine teeth 3. Dermosphenotic present. Frontal squamation E-patterned; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Cephalic neuromasts: supraorbital 4+3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 2+16–18, preorbital 3, otic 1, post-otic 2, supratemporal 1, preopercular 2+9–10, median opercular 1, ventral opercular 1–2, mandibular 8–9, lateral mandibular 3, paramandibular 1. Gill-rakers on first branchial arch 1–2+8. Six branchiostegal rays. Basihyal sub-triangular, width about 70 % of length in males, about 65 % in females; basihyal cartilage about 30 % of total length of basihyal in males, about 25 % in females. Ventral process of autopalatine present.

Dorsal fin rounded in both sexes, anal fin slightly pointed in males, rounded in females, caudal fin rounded to subtruncate in males, rounded in females; no filamen-

Table 1. Morphometric data of *Neofundulus rubrofasciatus*.

	holotype	paratypes	
	male	males (2)	females (4)
Standard length (mm)	43.3	32.4–33.6	30.7–33.8
Percent of standard length			
Body depth	23.4	24.2–24.5	24.3–25.7
Caudal peduncle depth	14.7	14.3–15.1	12.9–13.6
Pre-dorsal length	67.7	67.6–69.3	69.8–74.0
Pre-pelvic length	57.3	57.1–57.8	59.2–61.8
Length of dorsal-fin base	20.8	18.3–19.0	15.9–17.7
Dorsal-fin length	22.6	23.9–24.0	18.9–20.0
Length of anal-fin base	25.2	21.7–22.5	17.7–19.1
Anal-fin length	25.9	25.8–27.6	18.6–19.7
Caudal-fin length	37.2	36.7–38.0	34.5–36.4
Pectoral-fin length	28.5	27.0–29.6	23.4–26.0
Pelvic-fin length	15.6	14.9–15.5	10.8–11.9
Head length	26.2	26.9–29.0	26.6–28.4
Percent of head length			
Head depth	80.0	74.7–79.8	77.2–79.7
Head width	83.5	77.9–79.2	80.8–85.4
Snout length	13.4	16.0–17.6	13.9–14.6
Lower jaw length	20.6	16.1–19.3	16.4–18.1
Eye diameter	28.0	28.6–31.2	30.1–33.0

tous ray on unpaired fins. No scales on dorsal and anal fins, scales extending on about 30 % of caudal fin. Two neuromasts on caudal-fin base. Pectoral fin rounded, posterior margin reaching vertical between pelvic-fin base and anus in males, not reaching pelvic-fin base in females. No scales on pectoral-fin base. Pelvic-fin tip reaching between base of fourth and fifth anal-fin rays in males, between urogenital papilla and anal-fin origin in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin in vertical between base of fourth and fifth anal-fin rays. Dorsal-fin origin between neural spines of vertebrae 18 and 19; anal-fin origin between pleural ribs of vertebrae 16 and 17. Dorsal and ventral hypurals plates separated. Ventral process of posttemporal well-developed. Dorsal-fin rays 13–15; anal-fin rays 15–17; caudal-fin rays 24–27; pectoral-fin rays 14–15; pelvic-fin rays 7. No contact organ on fins.

Colouration. Males. Side of body light metallic blue to purplish blue, with eight horizontal rows of red or golden spots united, forming stripes narrower than interspace, rows 1, 7 and 8 usually not posteriorly reaching posterior-most portion of caudal peduncle, rows 2–6 reaching it; row 1 golden, rows 2, 4 and 6 red, rows 3 and 5 red anteriorly, golden posteriorly, rows 7 and 8 irregularly alternating red and golden spots. Large round dark grey to black humeral blotch, larger than orbit. Short bright yellow zigzag horizontal lines, placed between flank stripes, anteriorly and dorsally in contact with humeral blotch. Dorsum pale brown, venter bright orange. Side of head pale greenish golden. Iris light orangish yellow, with dark brown bar. Dorsal fin alternating four to six five dark red-

dish grey, almost black, transverse stripes and transverse rows of small pale white or orangish white spots. Anal fin dark reddish grey, almost black, with red basal stripe and bright yellow sub-basal stripe. Caudal fin dark grey, to black ventrally, with white or light orange sub-ventral stripe; red or light orange spots irregularly arranged on sub-dorsal and sub-posterior portions of fin. Pectoral fin with light greenish grey lines on middle to pale orange on borders, with dark grey dots on whole fin. Pelvic fin orange.

Females. Side of body pale brownish grey, with horizontal rows of dark brown spots, three central rows extending along whole flank, remaining rows posteriorly ending between vertical through pelvic-fin base and anterior portion of caudal peduncle; anterior portion of stripes nearly equal in width to interspace. Short pale greenish golden zigzag horizontal lines on anterior portion of flank, placed between flank stripes. Dorsum pale brown, venter white and yellow. Side of head brown with pale golden iridescence on opercle. Iris light yellow, with dark brown to black bar. Dorsal and anal fins pale white with three or four black transverse stripes, distal margin dark grey. Caudal fin pale white with four or five black transverse irregular bars, forming reticulate pattern on antero-dorsal and antero-ventral portions; posterior margin dark grey. Pectoral fin yellowish hyaline with dark grey dots. Pelvic fin yellowish hyaline.

Etymology. From the Latin *rubrofasciatus* (with red stripes), referring to the red stripes on the caudal peduncle and anal-fin base in males.

Distribution, habitat and taxonomical remarks. *Neofundulus rubrofasciatus* is known from two localities in the Miranda river drainage (Fig. 2), both comprising shallow seasonal pools, about 50 cm in the deepest places, with dense aquatic vegetation, in areas highly exposed to sunlight. The type locality, first sampled in July 2014, is situated in an almost plain area continuous to the floodplains of the Salobra river (20°29'42"S 56°48'37"W), a tributary of the Miranda river. The other locality, first sampled in March 1991, is situated in the Miranda river floodplains, near the town of Miranda (20°14'56"S 56°24'06"W). At that time, only two juvenile specimens (UFRJ 1012) and an adult male, about 35 mm SL, photographed in life but not preserved, were found. This site was later visited twice, in May 1996 and July 2014, but the whole region was highly deforested for agricultural proposals, and no specimen was found again in this area, as well as in other areas of the middle and upper Miranda river floodplains. No other killifish was collected in both localities.

Two preserved specimens listed in COSTA (1988) and previously identified as *N. paraguayensis*, from the Formoso river floodplains (about 20°09'S 56°17'W), also in the Miranda river drainage, possibly belong to this species, but identification confirmation depends on examination of larger samples and data on colouration in live individuals. These records suggest that *N. rubrofas-*

ciatus is geographically widespread along the Miranda river drainage and possibly it is not an endangered species, since the area around the type locality is still well-preserved.

Neofundulus aureomaculatus, new species

Fig. 4, Table 2

Holotype: UFRJ 10087, male, 46.6 mm SL; Brazil: Estado de Mato Grosso do Sul: Município de Aquidauana: temporary pool about 15 km NW of the town of Aquidauana, road MS-170, Aquidauana river floodplains, Paraguay river basin, 20°19'14"S 55°50'36"W, altitude about 135 m; W.J.E.M. COSTA *et al.*, 20 July 2014.

Paratypes: All from the Aquidauana river drainage, Paraguay river basin. Brazil: Estado de Mato Grosso do Sul: Município de Aquidauana: UFRJ 10088, 9 males, 34.2–42.2 mm SL, 10 females, 33.6–52.8 mm SL; UFRJ 10089, 2 males, 43.6–45.2 mm SL, 2 females, 34.4–36.6 mm SL (C&S); UFRJ 10090, 1 male, 35.5 mm SL, 2 females, 33.0–35.6 mm SL; collected with holotype. – UFRJ 3647, 4 males, 42.7–45.8 mm SL, 6 females, 36.0–43.9 mm SL; UFRJ 3648, 2 males, 39.0–44.8 mm SL, 2 females, 39.2–40.7 mm SL (C&S); temporary pool near Rancharia, about 70 km NW of the town of Aquidauana, road MS-170, 19°53'16"S 56°01'21"W, altitude about 125 m; W.J.E.M. COSTA *et al.*, 22 April 1996.

Diagnosis. Distinguished from all congeners by one character of the colour pattern in males, comprising the presence of alternating golden and red spots on the anterior portion of flank rows 3 and 4, with golden spots being twice deeper than neighbouring red spots (*vs.* golden spots, when present on anterior portion of flank rows 3 and 4, approximately equal in depth to neighbouring red spots). Also distinguished from all species, except *N. parvipinnis*, *N. rubrofasciatus*, and *N. splendidus*, by the presence of highly contrasting red or purple stripes and golden stripes on the caudal peduncle in males (*vs.* horizontal rows of dark reddish brown and pale yellow spots), and rows of golden spots placed between flank stripes on the anterior-most part of the flank in males (*vs.* golden spots absent); from all congeners, except *N. rubrofasciatus*, and *N. splendidus*, by the presence of a round black or dark grey humeral spot, larger than orbit at least in males (*vs.* vertically elongated, its horizontal width smaller than orbit); from *N. rubrofasciatus*, by having short straight rows of yellow spots on the anterior-most portion of the flank, between flank stripes (*vs.* bright yellow zigzag horizontal lines), basal portion of anal fin purple in males (*vs.* intense red), vertical series of golden spots on the anterior portion of the flank (*vs.* golden spots not arranged in vertical series), and scales extending over about 45–50 % of the anterior caudal fin in males (*vs.* about 30 %); from *N. parvipinnis* and *N. rubrofasciatus*, by having a longer lower jaw in males (lower jaw length 20.8–24.0 % of head length,



Fig. 4. *Neofundulus aureomaculatus*: A, UFRJ 10087, holotype, male, 46.6 mm SL; B, UFRJ 10088, paratype, male, 41.2 mm SL; C, UFRJ 10088, paratype, female, 38.9 mm SL; Brazil: Mato Grosso do Sul: Aquidauana.

vs. 17.4–19.4 % in *N. parvipinnis* and 16.1–20.6 % in *N. rubrofasciatus*); from *N. splendidus* by having 34–36 scales on the longitudinal series (vs. 40–41) and absence of black bars on the dorsal fin in males (vs. presence); from *N. parvipinnis* by having dorsal-fin base larger and dorsal fin slightly more anteriorly positioned in males (dorsal-fin base length 17.5–20.9 % SL,

vs. 13.7–16.9 % SL; pre-dorsal length 63.6–69.0 % SL, vs. 71.3–73.7 % SL; and dorsal-fin origin between base of anal-fin rays 2 and 4 vs. anal-fin rays 5 and 6) and head deeper in males (head depth 77.4–84.8 % of head length vs. 70.5–73.4 % head length); and from *N. paraguayensis* by having 16 rows of scales around caudal peduncle (vs. 20), 2+8–9 gill-rakers on the first branchial arch

(vs. 3 + 10–11), and dorsal and ventral hypurals plates separated (vs. united).

Description. Morphometric data appear in Table 2. Largest male examined 46.6 mm SL; largest female examined 52.8 mm SL. Dorsal profile gently convex and ventral profile approximately straight between snout and anterior part of caudal peduncle, nearly straight to caudal-fin base. Body slender, greatest body depth in vertical just anterior to pelvic-fin insertion. Urogenital papilla small, globular in males, urogenital opening placed in pocket-like structure in females. Longitudinal series of scales 34–36; transverse series of scales 10; scale rows around caudal peduncle 16. One neuromast on each scale of lateral line. No contact organ on scales. Total vertebrae 34–36.

Eye positioned on dorsal portion of head side. Snout short, slightly in males, blunt in females and juvenile specimens. Premaxilla and dentary teeth conical, small, numerous, irregularly arranged, except for external series with longer fang-like teeth, more robust and more curved inside mouth in males. Vomerine teeth 2–5. Dermosphenotic present. Frontal squamation E-patterned; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Cephalic neuromasts: supraorbital 4 + 3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 2 + 14–17, preorbital 3, otic 1, post-otic 2, supratemporal 1, pre-opercular 2 + 8–12, median opercular 1, ventral opercular 1–2, mandibular 8–11, lateral mandibular 2–4, paramandibular 1. Gill-rakers on first branchial arch 2 + 8–9. Six branchiostegal rays. Basihyal sub-triangular, width about 75 % of length in males, about 70 % in females; basihyal cartilage about 25–35 % of total length of basihyal. Ventral process of autopalatine present.

Dorsal fin rounded in both sexes, anal fin pointed in males, rounded in females, caudal fin rounded to subtruncate in males, rounded in females; no filamentous ray on unpaired fins. No scales on dorsal and anal fins, scales extending on about 45–50 % of caudal fin in males, about 30 % in females. Two neuromasts on caudal-fin base. Pectoral fin rounded, posterior margin reaching vertical between pelvic-fin base and anus in males, not reaching pelvic-fin base in females. No scales on pectoral-fin base. Pelvic-fin tip reaching between base of fourth and sixth anal-fin rays in males, between urogenital papilla and anal-fin origin in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin in vertical between base of second and fourth anal-fin rays. Dorsal-fin origin between neural spines of vertebrae 18 and 20; anal-fin origin between pleural ribs of vertebrae 16 and 18. Dorsal and ventral hypurals plates separated. Ventral process of posttemporal well-developed. Dorsal-fin rays 13–15; anal-fin rays 14–16; caudal-fin rays 26–29; pectoral-fin rays 14–15; pelvic-fin rays 7. No contact organ on fins.

Colouration. Males. Side of body light metallic blue, with eight horizontal rows of dark purple or golden spots united, forming stripes slightly wider than inter-

Table 2. Morphometric data of *Neofundulus aureomaculatus*.

	Holotype	paratypes	
	male	males (10)	females (10)
Standard length (mm)	46.6	34.2–45.8	32.3–52.8
Percent of standard length			
Body depth	25.3	23.7–26.4	22.3–25.7
Caudal peduncle depth	15.9	13.3–16.9	12.7–15.9
Pre-dorsal length	66.3	63.6–69.0	67.2–73.5
Pre-pelvic length	56.0	55.2–58.7	57.9–61.8
Length of dorsal-fin base	19.7	17.5–20.9	15.1–16.4
Dorsal-fin length	24.0	19.5–24.4	16.7–18.4
Length of anal-fin base	22.1	19.7–22.9	15.4–17.6
Anal-fin length	24.5	24.2–27.7	16.9–18.7
Caudal-fin length	34.4	36.7–38.0	31.4–34.9
Pectoral-fin length	26.4	26.1–30.3	22.5–25.6
Pelvic-fin length	—	16.0–20.3	10.7–12.3
Head length	27.9	24.9–29.0	26.1–28.6
Percent of head length			
Head depth	80.7	77.4–84.8	74.7–85.7
Head width	78.6	75.8–83.2	76.8–87.1
Snout length	14.6	13.2–15.7	12.3–14.7
Lower jaw length	23.9	20.8–24.0	16.9–21.2
Eye diameter	26.5	24.7–33.9	26.1–30.1

space, rows 1, 7 and 8 usually not posteriorly reaching posterior-most portion of caudal peduncle, rows 2–6 reaching it; row 1 golden, rows 2, 4 and 6 dark purple, rows 3, 5 and 7 with alternating large golden and small dark purple anteriorly, golden posteriorly, row 8 dark purple. Large round dark grey to black humeral blotch, larger than orbit. Short horizontal series of golden spots on anterior-most portion of flank, placed between flank stripes. Dorsum pale brown, venter bright orange. Side of head pale greenish golden. Iris light orangish yellow, with dark brown bar. Dorsal fin alternating four or five dark grey, almost black, transverse stripes and transverse rows of small pale white or yellowish white spots. Anal fin dark grey, almost black, with dark purple basal stripe and bright yellow sub-basal stripe. Caudal fin dark grey, to black ventrally, with white or light orange sub-ventral stripe; row of small white spots along sub-dorsal and sub-posterior portions of fin; sometimes pale orange posterior margin. Pectoral fin dark orangish brown with small white and dark grey spots. Pelvic fin orange.

Females. Side of body pale purplish grey, with horizontal rows of dark brown spots, three central rows extending along whole flank, remaining rows posteriorly ending between vertical through pelvic-fin base and anterior portion of caudal peduncle; anterior portion of stripes broader than interspace. Short pale golden horizontal lines on anterior portion of flank, placed between flank stripes. Dorsum pale brown, venter white. Side of head brown with pale golden iridescence on opercle. Iris light yellow, with dark brown to black bar. Dorsal and anal fins pale white with three or four black transverse stripes. Caudal fin pale white or yellowish white with five to seven black

transverse irregular bars, forming reticulate pattern on anterior portion. Pectoral fin yellowish hyaline with dark grey dots. Pelvic fin yellowish hyaline.

Etymology. From the Latin *aureomaculatus* (with golden spots), an allusion to the presence of numerous golden spots on the flank in males.

Distribution, habitat and taxonomical remarks. *Neofundulus aureomaculatus* was collected twice (Fig. 2). The first collection was made in a temporary pool about 70 km from the town of Aquidauana (19°53'16"S 56°01'21"W), in May 1996. Material from this locality was subsequently identified as *N. paraguayensis* (COSTA, 1998, 2014). The second collection was made in a temporary pool about 55 km south of the first one (20°19'14"S 55°50'36"W, the type locality), in July 2014. Both localities are situated close to a narrow road, poorly accessible during the rainy season, parallel and close to the Aquidauana river. The habitat consisted of small and shallow temporary pools (about 30 m in its larger length, about 0.5 m depth in deepest places), all individuals being collected close to margins, under dense aquatic and amphibious vegetation. No other killifishes were collected in these sites. The region is mainly occupied by large cattle farms and the original vegetation has been greatly removed, but seasonal pools have not been extirpated. There is no evidence suggesting that this species is presently endangered.

Neofundulus paraguayensis (Eigenmann & Kennedy, 1903)

Fig. 5

Fundulus paraguayensis EIGENMANN & KENNEDY, 1903: 530 (type locality: laguna [lagoon] near Arroyo Trementina, as indicated in the description, or aquadas and lagunitas [flooded area and pools] along Arroyo Trementina, a tributary of Río Aquido Canigi, as indicated in the list of localities of the same paper; holotype: [CAS-SU 42533](#), female, 43.5 mm SL).

Material examined: Paraguay river basin: Paraguay: [CAS-SU 42533](#), holotype; flooded area and pools along Arroyo Trementina, Río Aquido Canigi drainage; J.D. ANISITS. Brazil: [UFRJ 573](#), 42; [UFRJ 2113](#), 1 (C&S); [UFRJ 10082](#), 4 (C&S); Estado de Mato Grosso do Sul: Município de Porto Murinho: Nabileque river floodplains, Paraguay river basin, 20°38'50"S 57°31'37"W, altitude about 105 m; W.J.E.M. COSTA *et al.*, 20 March 1991. Argentina: [MLP 31-X-73-11](#), 4; Provincia Formosa: Departamento Bermejo: Ingeniero JUÁREZ; A. BACHMANN.

Diagnosis. Distinguished from all other species of the genus by having dorsal and ventral hypurals plates fused into a single plate (*vs.* separated). Also distinguished from all other species known to occur in the Pantanal

by having 20 rows of scales around caudal peduncle (*vs.* 16), 3+10–11 gill-rakers on the first branchial arch (*vs.* 2+8–9), and the presence of horizontal rows of dark reddish brown and pale yellow spots on the caudal peduncle in males (*vs.* highly contrasting red or purple stripes and golden stripes).

Distribution, habitat and taxonomical remarks. *Neofundulus paraguayensis*, as here delimited, occurs in a vast area of the Chaco biogeographical province and neighbouring transitional zones. It is here first recorded for the Brazilian Pantanal (Fig. 2), in the area known as Pantanal do Nabileque, a wide floodplain involving the Nabileque river and the main channel of the Paraguay river. Material from this locality was previously identified as *N. ornatipinnis* (COSTA, 1998), but comparison of type specimens suggests that both nominal species, *N. paraguayensis* and *N. ornatipinnis*, may be identical. This identification is tentative, since the only type specimen is presently poorly preserved, with fins severely damaged and scales lost in great part of body, besides topotypes have not been sampled. Consequently, contradictory data presented in the original description (EIGENMANN & KENNEDY, 1903) and in MYERS' type examination (1935) could not be checked. Examination of a x-ray image (available in http://researcharchive.calacademy.org/Image_db/IchTypes/Radio/Hi/42533-r.jpg) indicates that the anterior part of the dorsal fin is broken, remaining 12 proximal radials, which indicates a minimum of 14 dorsal-fin rays; there are 15 proximal radials in the anal fin, indicating the presence of at least 16 anal-fin rays; and the hypurals are fused, with dorsal and ventral plates limited by a vestigial fissure. All these characters are congruent with the population here identified as *N. paraguayensis*, from the southern end of the Pantanal, but limits between *N. paraguayensis* and *N. ornatipinnis* are unclear, as well as are also unclear the limits between these two nominal species and *N. acutirostratus*. The last species was described by COSTA (1992) on the basis of a single specimen ([CAS-SU 68326](#)), equivocally labeled as being collected by GEORGE MYERS in the upper São Francisco river basin, southeastern Brazil. Field studies conducted during the last decades have shown that there is no suitable habitats for the occurrence of seasonal killifishes in that region and probably the whole genus *Neofundulus* is geographically restricted to the central region of South America, in the Paraguay river basin and adjacent drainages of the Madeira river basin. The type specimen of *N. acutirostratus* exhibits morphological traits identical to specimens more recently collected in the western Paraguayan Chaco, including the prominent pointed snout, possibly being a synonym of *N. ornatipinnis*, if the latter is not a synonym of *N. paraguayensis*. In the Pantanal, *N. paraguayensis* was found in shallow (40 cm deep) seasonal pools with dense aquatic vegetation. Interestingly, this species has a unique behaviour among congeners occurring in the Pantanal, by jumping out water just after collector approximation, often keeping adhered to leaves just above surface of aquatic plants.



Fig. 5. *Neofundulus paraguayensis*, UFRJ 2113, male, 46.8 mm SL: Brazil: Mato Grosso do Sul: Porto Murtinho.

Discussion

The two species recognised as new in this study were found in neighbouring drainages, the Miranda and Aquidauana river drainages (Fig. 2). These river drainages have their headwaters situated in distant areas, the Miranda river having its upper section running from south to north, whereas the upper section of the Aquidauana river runs from northeast to southwest. In their lower section, just before the Aquidauana river flooding into the Miranda river, these drainages are connected by a complex plain aquatic system, formed by numerous permanent and temporary channels, which are locally known as corixos and vazantes, respectively, and small lagoons, known as baías. At the first glance, this drainage intersection would suggest a possible way for genetic flow between the two proposed species. However, interestingly, all records of species of *Neofundulus* for the Pantanal strongly indicates that all species are geographically restricted to the dryer sections of the drainages, beyond the core flooded zone, which seems to act as a natural barrier for *Neofundulus* dispersion. Species of *Neofundulus* have a peripheral distribution in the Pantanal (Fig. 2), not being recorded for the central intensely flooded zone. In addition, their habitat preference strongly contrasts with species of other seasonal killifish genera widespread in the Pantanal, *Pterolebias* and *Trigonectes*, which occur in temporary baías and vazantes, instead of shallow and isolated pools situated in dryer and slightly higher places, as those inhabited by species of *Neofundulus*.

Neofundulus rubrofasciatus and *N. aureomaculatus*, like *N. parvipinnis*, were found in habitats with the typical savannah-like vegetation of the central Brazilian Cerrado, consisting of tortuous shrubs with irregular and twisted ramifications, and coriaceous leaves. On the other hand, the single locality where *N. paraguayensis* was

collected in the Pantanal is a clear transition between the Cerrado and the Chaco, predominating the xerophytical deciduous vegetation of the latter, which is a semi-arid phytogeographical province occupying a vast portion of southern Bolivia, Paraguay and northwestern Argentina. Since a strong association between geographical distribution of seasonal killifishes and the limits of phytogeographical provinces have been broadly documented (COSTA, 1995b, 2002), this study indicates that *N. paraguayensis* is a species endemic to the Chaco, but geographically extending to the southern end of the Pantanal, where there is a clear Chacoan influence.

The three species endemic to the Cerrado areas of the Pantanal share a derived character state of the colour pattern in males, the presence of highly contrasting red or purple stripes and golden stripes on the caudal peduncle in males, not occurring in *N. paraguayensis* (Fig. 5). This character state colour pattern is also found in *N. splendidus*, a species endemic to the savannahs of the Llanos of Moxos, in the Mamoré river drainage of the Amazonas river basin (NIELSEN & BROUSSEAU, 2013), but never recorded among populations of *Neofundulus* reported to occur in the Chaco, suggesting that *N. rubrofasciatus*, *N. aureomaculatus*, *N. parvipinnis*, and *N. splendidus* are more closely related themselves than to the Chacoan congeners. These four species also have a round black humeral spot, which is larger than eye in *N. rubrofasciatus*, *N. aureomaculatus*, and *N. splendidus*, also suggesting a close relationship.

Some degree of polymorphism in the colouration of the caudal fin in males is here recorded for species of *Neofundulus*, thus not used as diagnostic features, although some variation may be informative to distinguish some species. The sub-marginal ventral stripe may appear in two colours, white to pale orangish white or bright red, in different individuals of the same population, but in *N. aureomaculatus* and *N. rubrofasciatus* that stripe is never bright red. On the other hand, the marks

of the dorsal portion of the fin may have different shapes and colours within a single population, but in 20 males of *N. aureomaculatus*, the colour pattern of the caudal fin comprised a series of small white spots along the sub-dorsal and sub-distal regions (Fig. 4a–b), a pattern not found in *N. rubrofasciatus*, which had irregularly arranged bright red or orange spots (Fig. 3–b).

Acknowledgements

Thanks are due R.P.D'Arrigo, M.A. Barbosa, F.A. Bockmann, C.P. Bove, B.B. Costa, E. Henschel, A. Katz, I. Landim, M. Melgaço, and C. Moreira for help during collecting trips; to L. Brandão for support in studies within the RPPN SESC-Pantanal; and to E.K. Bastos, L. Braga, H. Britski, D. Catania, N.A. Menezes, O. Oyakawa, L. Parenti, and S. Weitzman, for help in obtaining material, curatorial support or hospitality during visits to their institutions. This study was funded by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - Ministério de Ciência e Tecnologia) and FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro). Collections were made with licenses provided by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) and ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade).

References

- ARÁMBURU, A.S.A., ARÁMBURU, R.H. & RINGUELET, R.A. (1992): Peces paranaenses nuevos para la fauna argentina. – *Physis*, **23**: 223–239.
- COSTA, W.J.E.M. (1988a): Sistemática e distribuição do gênero *Neofundulus* (Cyprinodontiformes, Rivulidae). – *Revista Brasileira de Biologia*, **48**: 103–111.
- COSTA, W.J.E.M. (1988b): A new species of the neotropical annual fish genus *Pterolebias* (Cyprinodontiformes, Rivulidae), from central Brazil. – *Journal of Zoology*, **215**: 657–662.
- COSTA, W.J.E.M. (1988c): 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.
- COSTA, W.J.E.M. (1992): Descrição de uma nova espécie do gênero *Neofundulus* (Cyprinodontiformes: Rivulidae), da bacia do rio São Francisco. – *Revista Brasileira de Biologia*, **52**: 615–618.
- COSTA, W.J.E.M. (1995a): Two new genera and two new species of the neotropical annual fishes Plesiolebiathini (Cyprinodontiformes: Rivulidae), with studies on the relationships of the tribe. – *Revue Française d'Aquariologie*, **21**: 65–74.
- COSTA, W.J.E.M. (1995b): Pearl killifishes – the Cynolebiatinae: systematics and biogeography of the neotropical annual fish subfamily. Neptune City: TFH, 128p.
- COSTA, W.J.E.M. (1998): Phylogeny and classification of Rivulidae revisited: origin and evolution of annualism and miniaturization in rivulid fishes. – *Journal of Comparative Biology*, **3**: 33–92.
- COSTA, W.J.E.M. (2001): The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. – *Ichthyological Exploration of Freshwaters*, **12**: 333–383.
- COSTA, W.J.E.M. (2002): Peixes anuais brasileiros: diversidade e conservação. Curitiba: Editora UFPR, 238p.
- COSTA, W.J.E.M. (2003): *Moema heterostigma*, a new annual fish (Cyprinodontiformes: Rivulidae) from the Brazilian Pantanal, Rio Paraguay basin. – *Ichthyological Exploration of Freshwaters*, **14**: 289–294.
- COSTA, W.J.E.M. (2005): The Neotropical annual killifish genus *Pterolebias* Garman (Teleostei: Cyprinodontiformes: Rivulidae): phylogenetic relationships, descriptive morphology, and taxonomic revision. – *Zootaxa*, **1067**: 1–36.
- COSTA, W.J.E.M. (2007): Taxonomy of the plesiolebiasine killifish genera *Pituna*, *Plesiolebias* and *Maratecoara* (Teleostei: Cyprinodontiformes: Rivulidae), with descriptions of nine new species. – *Zootaxa*, **1410**: 1–41.
- COSTA, W.J.E.M. (2014): Phylogeny and evolutionary radiation in seasonal rachovine killifishes: biogeographical and taxonomical implications. – *Vertebrate Zoology*, **64**: 177–192.
- DAVIS, J.I. & NIXON, K.C. (1992): Populations, genetic variation, and the delimitation of phylogenetic species. – *Systematic Biology*, **41**: 421–435.
- EIGENMANN, C.H. & KENNEDY, C.H. (1903): On a collection of fishes from Paraguay, with a synopsis of the American genera of cichlids. – *Proceedings of the Academy of Natural Sciences of Philadelphia*, **55**: 497–537.
- HOEDEMAN, J.J. (1958): The frontal scalation pattern in some groups of toothcarps (Pisces, Cyprinodontiformes). – *Bulletin of Aquatic Biology*, **1**: 23–28.
- MYERS, G.S. (1935): Four new fresh-water fishes from Brazil, Venezuela and Paraguay. – *Proceedings of the Biological Society of Washington*, **48**: 7–14.
- MYERS, G.S. (1942): Studies on South American fresh-water fishes. I. – *Stanford Ichthyological Bulletin*, **2**: 89–114.
- NIELSEN, D.T.B. & BROUSSEAU, R. (2013): Description of a new species of annual fish of the genus *Neofundulus* (Cyprinodontiformes: Rivulidae) from the upper rio Mamoré basin, Bolívia. – *Aqua International Journal of Ichthyology*, **19**: 109–114.
- ROSEN, D.E. (1979): Fishes from the uplands and intermontane basins of Guatemala: revisionary studies and comparative geography. – *Bulletin of the American Museum of Natural History*, **162**: 267–376.
- SANTOS F., P.S. (1979): The South American annual fish *Neofundulus paraguayensis* (Eigenmann and Kennedy, 1903). – *Journal of the Killifish Association*, **12**: 33–40.
- TAYLOR, W.R. & VAN DYKE, G.C. (1985): Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. – *Cybium*, **9**: 107–109.