Re-description and distribution of *Rivulus punctatus* Boulenger, 1895 (Teleostei: Rivulidae) and its habitats in Paraguay

Ingo Schindler [1] & Vollrad Etzel [2]

[1] Warthestr. 53a, 12051 Berlin, ingoschindler@web.de
[2] Hamburg-Amerika-Str. 6; 27472 Cuxhaven

Received on December 19, 2007, accepted on February 18, 2008.
Published online at www.vertebrate-zoology.de on May 12, 2008.

> Abstract

Based on specimens collected during several field trips in Paraguay the morphological variation, habitat requirements, and distribution of the rivulide killifish *Rivulus punctatus* from río Paraguay drainage are described (including specimens from the vicinity of the type locality). Males differ from females in differences of the colour pattern in the unpaired fins and in morphometrics (including longer pelvic fins and shorter preanal-fin length). The conspicuous variation in the caudal fin pattern of males is significantly correlated with the standard length ($p<0.01$). There is no correlation between morphometric data or caudal-fin pattern and geographic localities (in both cases $p>0.05$). The habitats are characterized by a low depth (usually less than 30 cm) of water, slow or no current and usually plenty of water and marsh plants or flooded bushes and grass.

> Resumen

En base a ejemplares que fueron recolectados durante varios viajes al Paraguay, se describen la morfología, las caracteristicas de habitat y la distribución geográfica de *Rivulus punctatus*, en el sistema fluvial del río Paraguay (incluyendo ejemplares de las cercanias de la localidad tipo). Los machos se diferencian de las hembras en la coloración de las aletas impares y en las morfometrias (entre otras caracteristicas, aletas ventrales mas largas y una longitud preanal mayor). La variación notable en el diseno de la alteta caudal del macho esta correlacionada significativamente con la longitud estándar ($p<0,01$). No se ha podido establecer una correlación entre los datos morfometricos o el diseno de la aleta caudal con la situación geografica del sitio de recolección (en ambos casos $p<0,05$). Los habitat se caracterizan por una escasa profundidad del agua, velocidad de caudal mas reducida o carente y numerosas plantas acuáticas, del pántano o arbustos y pastos cubiertos por el agua.

> Kurzfassung

Anhand von Exemplaren, die während verschiedener Reisen in Paraguay gesammelt wurden, werden die Morphologie, die Habitatansprüche und Verbreitung des rivulinen Killfisches *Rivulus punctatus* aus dem Flusssystem des Rio Paraguay beschrieben (einschließlich von Exemplaren aus der Nähe der Typuslokalität). Männchen unterscheiden sich von den Weibchen in der Färbung der unpaaren Flossen und in der Morphometrie (u.a. längere Bauchflossen und eine größere Praeanallänge). Die auffällige Variation der Schwanzflossenmusterung der Männchen ist signifikant mit der Standardlänge korreliert ($p<0,01$). Es konnte keine Korrelation zwischen morphometrischen Daten oder dem Schwanzflossenmuster und der geographischen Lage des Fundplatzes ermittelt werden (in beiden Fällen $p>0,05$). Die Habitate sind durch eine geringe Wassertiefe, geringe oder keine Fließgeschwindigkeit und zahlreiche Wasser- und Sumpfpflanzen oder überflutete Büsche und Gräser gekennzeichnet.

> Key words

Killifishes, Neotropics, Taxonomy, Ecology, Distribution, Paraguay, Rivulini, *Rivulus punctatus*.

Introduction

The neotropical killifish genus *Rivulus* Poey, 1860 of the aplocheiloid family Rivulidae (subfamily Rivulinae) comprises more than 100 species (Costa, 2003a). Members are distributed from Middle-America and the Caribean Islands to northern Argentina and occur in a variety of habitats ranging from savannah and forest creeks, small ponds, swamps and temporary ditches. Based on morphological data the genus is recognized as a monophyletic assemblage (Huber 1999; Costa

2004, 2006). However, in a phylogenetic context it is poorly defined among the basal lineages of the Rivulidae (COSTA, 2004, 2006a, 2006b). In analyses of molecular data it turned out as paraphyletic (HRBEK & LARSON, 1999; HRBEK *et al*., 2004). Nevertheless, *Rivulus* contains well-supported monophyletic subunits, which are currently recognized as subgenera (HUBER, 1999; COSTA, 2006). One of these subgenera is the recently described *Melanorivulus* COSTA (2006). *Melanorivulus* includes the species of the *R. punctatus*-group (COSTA, 1995). This monophyletic unit comprises about 20 species (COSTA, 2005, 2006a, 2006b, 2007a, 2007b). The type species *R. punctatus* is the most southern species in *Melanorivulus*. It is distributed in the río Paraguay, lower río Paraná and middle río Uruguay drainage in Brazil, Bolivia, Paraguay and Argentina (COSTA, 1995).

Rivulus punctatus was described by BOULENGER (1895) on the basis of specimens collected at Colonia Risso in Paraguay. Thus it is the oldest taxon within the subgenus. The species was already the subject of taxonomic treatments (HUBER, 1992; STAECK & SCHINDLER, 1994; COSTA, 1995). However, none of these focused on specimens from Paraguay. During five field studies of the second author between 1984 and 2006 it was possible to catch several samples of *R. punctatus* in Paraguay, even in the vicinity of the type locality. Although there are several collecting sites of *R. punctatus* in Paraguay listed (e. g. HUBER, 1992, 1995; ETZEL, 1985, 1991), none of them contains a description of its ecology and habitats. Therefore the objectives of the present article are to describe *R. punctatus* from Paraguay, to analyse its variation and to document the habitats of this species.

Methods

Measurements and counts follow HUBER (1992). He and the majority of authors (e. g. THOMERSON & TAPHORN, 1992; LASSO-ALCALÁ *et al*., 2006) use the snout tip as the most anterior point of reference. COSTA (1988), however, used the middle of the posterior limit of the depression between upper jaw and the neurocranium, and his morphometric data are compatible only to a certain extent with those published here. Measurements were made with an electronic calliper reading to the nearest 0.1 mm. Fin-ray counts were made with light transmitted through the fins and included all discernible elements. Using this method the counting of the number of fin rays in alcohol specimens is not without difficulties, and it may happen that sometimes the very minute anterior rays of anal- dorsal- or caudal-fin are overlooked and not counted (see HUBER, 1992).

The material of *R. punctatus* from Paraguay used is listed under species description (see also Table 1). The majority of the specimens are very small, weak or otherwise not well enough preserved. Therefore 20 representative specimens (10 males and 10 females) are selected to obtain the morphometric and meristic data. Additional counts from four cleared & stained specimens are included. Terminology of colour pattern, shapes and frontal squamation follow HUBER (1992). In order to make comparison of the species easier, the description follows the general format used by COSTA (2005).

Principal components analysis was applied to investigate patterns of sexual dimorphism in multidimensional space and in order to visualize the pattern of variation in morphometric data along geographic locations. The geographic locality is represented by scores on the first PCA axis obtained from the untransformed decimal equivalents of latitude and longitude of the collecting sites of each included specimens (see WOODMAN, 2000 for detailed description of this procedure). Two separate PCA were calculated for the geographic position: first one with the localities of 20 specimens (10 males, 10 females) used for morphometric analysis (factor loadings on PC1: latitude = -0.989 and longitude = 0.148) and a second with localities of 27 males used for the coding of the caudal-fin pattern (factor loadings on PC1: latitude = 0.994 and longitude = -0.103). The PC1 axes accounts for 95.4% (first analysis) and 88.2% (second analysis) of variance respectively. Thus the scores reliably describe the relative geographic position of the localities (cf. Woodman, 2000). To correct the effect of size all measurements were standardized by expressing them as proportions of standard length as recommended by VAN VELZEN *et al*., (1992). For this purpose the equation $\log(x') = \log(SL) - \log(x)$ is used (BLACKITH & REYMANT, 1971). To make the caudal-fin pattern in males available for statistic analyses, it is coded into five stages (see description below). Since this character (stages are treated as ordered) is correlated with SL (Fig. 14), the codes are size adjusted for further statistic evaluation (Fig. 16) by subtraction of a linear regression line from the code values (although the correlation between un-adjusted codes of caudal-fin pattern and geographic locality is similar).

PCA (using the variance-covariance matrix), correlation coefficients and regressions lines were calculated with the programme PAST version 1.71 (HAMMER *et al*., 2001, 2007). For assumptions, necessary data and the descriptions of the statistic applications see HAMMER & HARPER (2006). Results are visualized using the programme KyPlot (YOSHIOKA, 1997-2000). The significance of pairwise differences between males and females in the one-dimensional standardized data (expressed as percent of SL) is tested with the *t*-test

as described in Lozan (1990). The p-values were corrected for multiple tests by the sequential Bonferroni-correction (see Schindler, 2003).

Abbreviations

ESC	Evolutionary Species Concept
HL	head length
MTD F	Staatliche Naturhistorische Sammlungen Dresden, Museum für Tierkunde, Fischsammlung
PCA	principal component analysis
SL	standard length
TL	total length.

Rivulus (*Melanorivulus*) *punctatus* Boulenger, 1895

(Tables 1–2, Figs 1–7)

Materials. MTD F 31225–29, 5 Ex., 21.3–30.3 mm SL; Paraguay, Belen, 23.4°S/57.2°W, Py 9/99; Etzel & Hessfeld *leg*., 25.11.99. Uncat., 15 Ex., 14.2–20.5 mm SL; Paraguay, Pilar, 57.7°S / 26.8°W, Py 2/99; Etzel & Hessfeld *leg*., 17.11.99. MTD F 31230–39, 10 Ex., 18.2–22.4 mm SL; Paraguay, La Rosada, 26,1°S/56,8°W, Py 1/01; V. Etzel & G. Hessfeld leg., 4.11.01. MTD F 31240–41, 2 Ex., 20.4-22.3 mm SL; Paraguay, Chaco-I, 25.2°S/57.6°W, Py 10/01; Etzel & Hessfeld *leg*., 12.11.01. MTD F 31242–46, 5 Ex., 18.2–26.3 mm SL; Paraguay, Sholollo, 25.5°S/57.0°W, Py 2/01; Etzel & Hessfeld *leg*., 5.11.01. MTD F 31247, 1 Ex., 29.2 mm SL; Paraguay, Bridge at Rio Salado, 25.3°S/57.5°W, Py 24/06; Etzel & Hessfeld *leg*., 19.11.06. MTD F 31248–51, 4 Ex., 25.5–31.0 mm SL; Paraguay, Conception, 23.4°S/57.5°W, Py 9/01; Etzel & Hessfeld *leg*., 16.11.01. MTD F 31252–62, 11 Ex., 18.9–24.1 mm SL; Paraguay, Belen, 23.4°S/57.2°W, Py 9/99; Etzel & Hessfeld *leg*., 25.11.99. MTD F 31263–64, 2 Ex., 27.9–29.2 mm SL; Paraguay, Belen, 23.4°S/57.2°W, Py 9/99; Etzel & Hessfeld *leg*., 25.11.99. MTD F 31265–70, 6 Ex., 19.0-25.4 mm SL; Paraguay, 20 km to Pilar, 26.8°S/58.2°W, Py 4/01; Etzel & Hessfeld *leg*., 8.11.01. MTD F 31271, 1 Ex., 25.6 mm SL; Paraguay, Rio Piribury, 25.1°S/57.25°W, Py 15/04; Etzel & Hessfeld *leg*., 21.11.04. MTD F 31272–77, 6 Ex., 23.7–32.6 mm SL; Paraguay, Paso Barreto, 22.8°S/57.2°W, Py 18/06; Etzel & Hessfeld *leg*., 14.06.06. MTD F 31278–84, 7 Ex., 17.7–25.9 mm SL; Paraguay, San Ignacio, 26.9°S/57.0°W, Py 6/01; Etzel & Hessfeld *leg*., 9.11.01. MTD F 31285–91, 7 Ex., 21.5-26.1 mm SL; Paraguay, Ayolas, 27.3°S/ 56.7°W, Py 5/01; Etzel & Hessfeld *leg*., 9.11.01. MTD F 31292–94, 3 Ex., 22.3–26.5 mm SL; Paraguay, Loreto, 23.3°S/57.3°W, Py 16/01; Etzel & Hessfeld *leg*.,16.11.01. MTD F 31295–96, 2 Ex., 25.6–28.7 mm SL; Paraguay, Trinidad/Jesus, 27.1°S/55.7°W, Py 1/99; Etzel & Hessfeld *leg*., 16.11.99. MTD F 31297–31306, 10 Ex., 21.6–33.1 mm SL; Paraguay, Conception, 23.4°S/57.5°W, Py 17/01; Etzel & Hessfeld *leg*., 16.11.01. MTD F 31307, 1 Ex., 28.1 mm SL; Paraguay, San Carlos, 25.9°S/56.2°W, Py 20/06; Etzel & Hessfeld *leg*., 17.11.06. MTD F 31308–10, 3 Ex., 28.2–35.1 mm SL; Paraguay, Pilar, 26.8°S/57.6°W, Py 24/01; Etzel & Hessfeld *leg*., 24.11.01. MTD F 31311, 1 Ex., 24.4 mm SL; Paraguay, Rio Piribury, 25.1°S/57.25°W, Py 15/04; Etzel & Hessfeld *leg*., 21.11.04.

Diagnosis

Type species of the subgenus *Melanorivulus* Costa, 2006. It is distinguished from the remaining members of the subgenus by the combination of the following character states: (1) caudal fin in both sexes spatula shaped, elongated longitudinally (versus caudal fin round, not particularly elongated), (2) red dots on body flanks not arranged in complete bars nor in longitudinal stripes (versus red dots arranged in chevron-like, oblique bars, longitudinal stripes or flanks of body plain), (3) scales in longitudinal series 30–32 (versus >31 scales), (4) no red bars, no orange stripe or yellow pattern on caudal fin (versus red bars, orange stripe or yellow pattern present), and (5) gill rakers on first arch 1+8 (versus 1+6).

Description

The description is based on specimens from Paraguay (Table 1). Morphometric data are summarized in Table 2. For general appearance see Figs. 1–7. Osteological data based on cleared & stained specimens only. Meristic data include counts of 26 specimens from Paraguay (number in parentheses after counts indicate the data of four cleared & stained specimens).

Maximum recorded adult size in males 35.2 mm SL, in females 30.9 mm SL. Dorsal profile slightly convex from snout to end of dorsal-fin base, straight on caudal peduncle. Ventral profile of head convex, almost straight or slightly convex between head and posterior end of anal-fin base; on caudal peduncle slightly concave. Body slender, subcylindrical anteriorly, slightly deeper than wide, posterior of dorsal-fin origin laterally compressed. Greatest body depth at pelvic-fin base. Dorsal and anal fin slightly pointed in males, round in females. Caudal fin horizontally elongated, posterior margin round. Pectoral fins rounded, 19.6% of SL in males, 18.8% of SL in females. Pelvic fins slightly pointed, reaching between urogenital papilla and anterior anal-fin base in males, and between anus and urogenital opening in females. Dorsal-fin origin on vertical through base of 10th or 11th anal-fin ray. Dorsal-fin rays 7, 8(4) or 9; anal-fin rays 13(2) or 14(2); caudal-fin rays 28(1), 29(2) or 30(1); pectoral-fin rays 12–13; pelvic-fin rays 6–7. Scales large, cycloid; basal radii 18–22. Body scales extending over caudal-fin base (about 30% of caudal-fin length), posterior margin of the scaled area convex. No scales on dorsal- and anal-fin bases. Frontal squamation F-patterned; rarely E-patterned. Scales in longitudinal series 30 to 32; transverse series 8 to 9; 22 to 24 predorsal scales. Gill


1
2
3
4
5
6
7

rakers on first arch 8 or 9. Vertebrae 29(1) or 30(1). Dorsal portion of preopercle comparatively short and pointed. Vestigal ventral process of angulo-articular not particularly developed. First epibranchial moderate (in younger specimens < 15 mm SL) to strongly bent (in adult specimens > 25 mm SL). Hypural forms two plates separated by a median gap. Epural and parhypural thin in smaller specimens and broad in larger specimens.

Both sexes differ significantly in univariate and multivariate morphometrics. Females possess a proportional larger preanal ($p<0.001$), prepelvic length ($p<0.05$), males distinguished by larger pelvic- ($p<0.01$) and anal-fin length ($p<0.05$). A multivariate analysis of eleven log transformed and size adjusted measurements (table 1) separate both groups (Fig. 13).

Colouration

Males. Sides of body metallic bluish-green (yellowish brown or greyish in alcohol) with dark reddish dots arranged into oblique chevron-like bars (number of red dots and arrangement is variable). Laterodorsal brown spots on anterior portion of body; dark grey longitudinal stripe between eye and caudal-fin base. Dorsum dark grey, venter white. Postorbital region metallic bluish-green. Chin and ventral part of head whitish. Lower jaw dark grey. Dorsal fin light bluish with red dots or thin reddish lines in the posterior parts. Anal fin light bluish with red dots and/or reddish oblique bars in posterior parts; margin with black stripe. Dark pattern on caudal fin very variable. Five different patterns on caudal fin of males are recognized. The following descriptions can give only a rough impression of the typical patterns, because there are variations within and continuities between several classes of caudal-fin patterns: Caudal fin with (1) vertical bars (mostly distally less prominent), (2) with bars interrupted in dorsal and ventral parts, or with scattered vertical lines, (3) dotted, (4) reticulated or (5) without conspicuous dark pattern. There is a significant correlation between SL and the coded caudal-fin patterns ($r=0.49$, $p<0.01$), for larger specimens tend to posses the caudal-fin patterns 3, 4 or 5 (the latter two states are observed in adult ones only).

Females. Sides of body grey to slightly purple with faint brown to dark longitudinal stripe and reddish dots. Dorsal, anal and pelvic fins with dark stripe on distal margin. Entire caudal fin with dark border; centre of caudal fin with blotchy spots, reticulated or marbled in larger specimens. Caudal spot present, slightly apart from dorsal margin (sometimes masked by marbled pattern or dark blotches).

Distribution

The species is widely distributed in the río Paraguay, lower río Paraná and lower río Uruguay drainages in Brazil, Bolivia, Paraguay and Argentina (Regan, 1912; Huber, 1992, 1995; Costa, 1995, 2003a; Staeck & Schindler, 1994). The records from the rio Guaporé basin (Henn, 1916) and near Porto Alegre (Huber, 1992) are questionable and need further confirmation (Costa, 1995).

In Paraguay *R. punctatus* is reported from Colonia Risso (terra typica) in the north (río Paraguay drainage) to Ayolas in the south (río Paraná drainage) and from San Juan Neomucero in the east to Pozo Colorado in the west, that means a maximal distance of 200 to 300 km from the río Paraguay channel. Central and western Paraguay is mainly characterized by the wide, hot and semi-arid lowlands (only up to 200 m asl) of the Chaco region. The Paraneña region (east of the río Paraguay), a mixture of plateaus and valleys, raises up to about 400 to 850 m asl (Cordillera de Amambay). Until now *R. punctatus* is only found in the lowlands (see Table 1 for the collecting data obtained by the second author).

Habitat

Rivulus punctatus occurs in the lowlands of Paraguay and prefers both flooded regions near the río Paraguay

Fig. 1. *Rivulus punctatus*; male, TL ≈ 39 mm; Py18/06, near to the type locality, see Table 1.

Fig. 2. *Rivulus punctatus*; male, TL ≈ 42 mm; Py18/06, near to the type locality, see Table 1.

Fig. 3. *Rivulus punctatus*; male, TL ≈ 38 mm; Py24/06, see Table 1.

Fig. 4. *Rivulus punctatus*; male, TL ≈ 34 mm; Py04/01, see Table 1.

Fig. 5. *Rivulus punctatus*; female, TL ≈ 41mm; Py09/99, see Table 1.

Fig. 6. *Rivulus punctatus*; female, TL ≈ 30 mm; Py16/01, see Table 1.

Fig. 7. *Rivulus punctatus*; female, TL ≈ 30 mm; Py04/01, see Table 1.

Tab. 1. Locations, habitat characteristics and examined specimens of *Rivulus punctatus* in Paraguay. n1 = number of specimens in the jar, n2 = number of specimens used in the morphometric data analyses, n3 = number of males used in the caudal-fin pattern analyses, μS = electric conductivity (μS/cm).

location No.	location	date	swamp/ brook	temperature (°C) air / water		pH	μS	coordinates	n1	n2	n3
Py 1/84	Itacurubi	24.06.84	brook	28.0	23.1	6.8		25.4°/56.8°			
Py 2/84	Eusebio Yala	24.06.84	brook	27.8	23.5	7.0		25.3°/56.9°			
Py 1/99	Trinidad/Jesus	16.11.99						27.1°/55.7°	2	1	
Py 2/99	50 km to San Ignatio	17.11.99	swamp	28.5	29.0	6.8	90	26.9°/57.5°	15		
Py 6/01	5 km to San Ignatio	09.11.01	brook	26.7	24.9	5.5	90	26.9°/57.0°	6	1	1
Py 8/01	Bautista	11.11.01	brook	29.8	24.5	5.8	102	26.7°/57.2°			
Py 1/01	La Rosada	04.11.01	brook	28.6	24.4	5.8		26.1°/56.8°	9		3
Py 3/04	San Carlos	07.11.04	swamp	29.3	20.5	5.8	39	25.9°/56.2			
Py 20/06	San Carlos	17.06.06	swamp	31.2	21.0	5.6		25.9°/56.2°	1	1	1
Py 2/01	Sholoło	05.11.01	brook	33.4	32.0	5.5		25.5°/57.0°	5		1
Py 8/99	20 km to Conception	24.11.99	swamp	37.9	38.3	7.4	530	23.3°/57.7°			
Py 8a/99	Conception	24.11.99	swamp	36.3	36.2	7.0	120	23.4°/57.5°			
Py 9/01	Conception	16.11.01	swamp	38.1	30.1	6.5	420	23.4°/57.5°	4	3	2
Py 8/04	Conception	11.11.04	swamp	31.8	26.3	5.8	130	23.4°/57.5°			
Py 17/01	Conception	16.11.01	swamp					23.4°/57.5°	10	1	
Py 16/01	Loreto	15.11.01	swamp	22.3	22.1			23.2°/57.2°	3		1
Py 5/04	Loreto	8.11.04	swamp					23.2°/57.2°	1		1
Py 18/06	Paso Barreto	14.06.06	brook	23.4	21.0	5.8		22.8°/57.2°	6	3	2
Py 9/99	Belen	25.11.99	brook	20.2	22.3	5.0	94	23.4°/57.2°	7	4	5
Py 9/06	Belen	14.06.06	brook	24.2	20.1	6.5		23.4°/57.2°			
Py 4/01	20 km to Pilar	08.11.01	swamp	39.2	30.8	6.1		26.8°/58.2°	16		3
Py 14/04	20 km to Pilar	19.11.04	swamp	29.7	29.3	5.8	47	26.8°/58.2°			
Py 24/01	Pilar	24.11.01						26.8°/57.6°	3	2	1
Py 5/01	3 km to Ayolas	09.11.01	swamp	30.0	29.8	5,8	107	27.3°/56.7°	7	3	4
Py 10/01	Falcon / Chaco-i	12.11.01	swamp	41.5	26.2	5,8	180	25.2°/57.6°	2		1
Py 15/04	Limpio	21.11.04	swamp	28.4	35.0	5,8	69	25.3°/57.5°	1		
Py24/06	Limpio	20.06.06	swamp	30.6	26.1	6,2		25.3°/57.5°	1	1	1

channel and creeks in (at least formerly) forest areas. It is difficult to determine now the original structure of some regions in Central-Paraguay, because at present most of them are deforested and used for extensive agriculture (mostly cattle-breeding, but also soybean, maize and sunflower plantations). There is the impending danger that even the rests of the primary forest will disappear in the next decade if the speed of denaturalization is not reduced.

Rivulus punctatus occurs in three typical types of habitats: firstly the more or less flooded margins of creeks and rivers with submerged vegetation (grass, bush and frequently *Nymphea*) at the embankments (Fig. 8 and 9, locations "La Rosada" and "Loreto"). In this kind of habitat *R. punctatus* was observed only 2 to 5 cm under the surface of the water between flooded grass and bushes. Sometimes it was also found in small ponds or puddles which remained after heavy rainfalls.

The second types of habitats (Fig. 10, location "Ayolas") are swamps as they are found in southern Paraguay between San Ignacio and Pilar. In this region the

Fig. 8. Habitat of *R. punctatus* near LaRosade, Py01/01, see Table 1.

Fig. 9. Habitat of *R. punctatus* near Loreto, Py16/01, see Table 1.

Fig. 10. Habitat of *R. punctatus* near Ayolas, Py05/01, see Table 1.

Fig. 11. Marsh land near of the type locality; habitat of *R. punctatus*, see Table 1.

Fig. 12. Collecting site of *R. punctatus* in the Gran Chaco near to Concepcion, see Table 1.

expansion of the swampy areas is extensive. Usually their bottom is muddy and the water clear and shallow (deepness approximately 20 to 50 cm). A lot of different aquatic or semi-aquatic plants like *Azolla*, *Ludwigia*, *Nymphea* and *Pistia* inhabit the swamps (see Fig. 11). There *Rivulus punctatus* was found – mostly sympatric with *Apistogamma borelli* – 2 to 3 cm below the surface of the water between the plants.

The third type of habitat of *R. punctatus* is found in the Chaco Paraguayo (Fig. 12, Chaco near Concepcion). In the dry season (i. e. the winter from June to September) small rivers and creeks often dry up, and only small pools with murky grey to brownish-red water are left over. In these habitats *R. punctatus* is accompanied by *Trigonectes balzanii*. Data of important water parameters are summarized in Table 1.

Discussion

Rivulus punctatus is the type species of *Melanorivulus* Costa, 2006 and therefore its subgeneric arrangement is without question. This subgenus was formerly known as the *punctatus*-superspecies (Huber, 1992) or as the *punctatus*-complex (Costa, 1995, 2005).

The diagnoses published for the taxon *R. punctatus* neither acknowledge the variation in colour pattern (cf. Costa, 1995) nor take into account the most closely related taxa (e.g. Huber, 1992; Staeck & Schindler, 1994). All earlier publications suffer from the lack of specimens collected at or near to the type-locality. This is why we provide here an evaluated diagnosis based on specimens of *Rivulus punctatus* from Paraguay. As

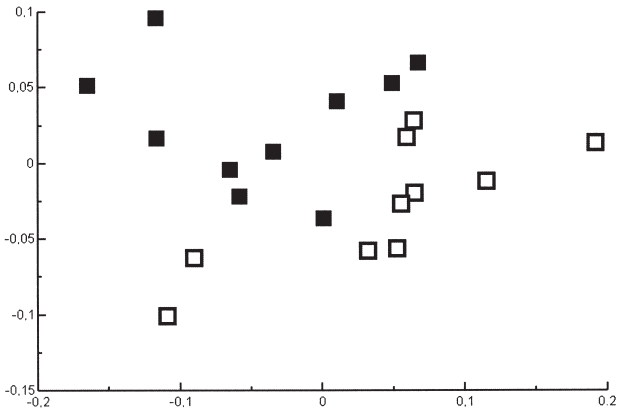

Fig. 13. Scatter-plot of factor scores (PCA of 11 measurements) for *R. punctatus* from Paraguay. PC1 (x-axis) accounted for 58.5% and PC2 (y-axis) for 16.9% of variance. Males = open symbols, females = filled symbols.

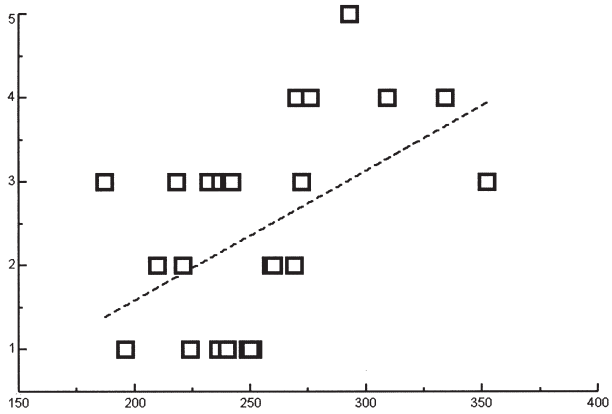

Fig. 14. Relationship between standard length in mm (x-axis) and codes of caudal-fin pattern (y-axis) using 27 males of *R. punctatus* from Paraguay. Dashed line = linear regression (a = 0.015, b = -1.514; r = 0.49, $p < 0.01$).

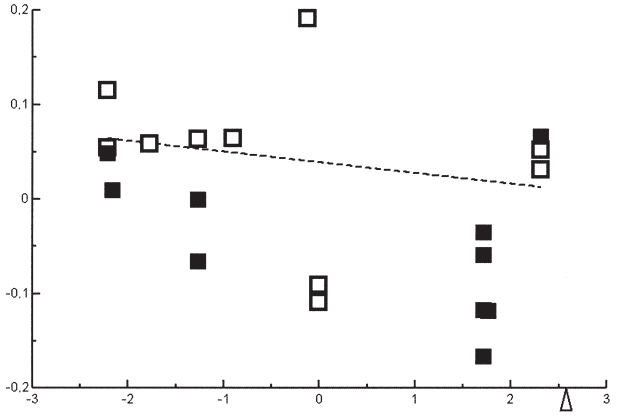

Fig. 15. Relationship between "geographic locality" (x-axis) and PC1 scores (y-axis) of morphometric data (10 males and 10 females) of *R. punctatus* from Paraguay. Dashed line = linear regression (a = -0.052, $b \approx 0$; r = -0.389, $p > 0.08$). Males = open symbols, females = filled symbols. Triangle at the abscissa indicates the approximate position of the type locality.

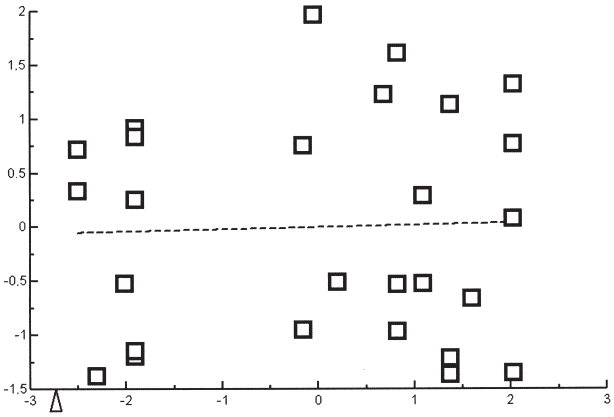

Fig. 16. Relationship between "geographic locality" (x-axis) and size-adjusted (residuals from a regressions with SL) codes of caudal-fin pattern (y-axis) using 27 males of *R. punctatus* from Paraguay. Dashed line = linear regression (a = 0.063, $b \approx 0$; r = 0.033, $p > 0.8$). Triangle at the abscissa indicates the approximate position of the type locality. Regression line and correlations coefficient for un-adjusted data (not shown) are: a = -0.724, b = 2.407 and r = -0.077, $p > 0.7$.

it is the oldest taxon (described by BOLENGER, 1895) within *Melanorivulus*, it is not in the danger of being regarded as a junior synonym of another taxon.

There are four further species of the subgenus *Melanorivulus* (viz. *R. cyanopterus* COSTA, 2006, *R. dapazi* COSTA, 2006, *R. bororo* COSTA, 2007 and *R. paresi* COSTA, 2007) distributed in the upper río Paraguay drainage (COSTA, 2006). *Rivulus cyanopterus* is differentiated from *R. punctatus* by possessing comparatively broad oblique red bars on the anal fin in both sexes and by the lack of distinctive colours or dark patterns in the caudal fin. *R. punctatus* is distinguished from *R. dapazi* by less scales in the longitudinal series (30–32 in *R. punctatus* vs. 34–35 in *R. dapazi*), by oblique bars of red dots on the sides of the body vs. chevron-like red lines in *R. dapazi* and by the lack of dark orange stripes on the caudal fin. *Rivulus bororo* and *R. paresi* differs from *R. punctatus* by a pattern of red dots on flank, arranged in chevronlike series. Further *R. bororo* is distinguished from *R. punctatus* by a fewer number of mandibular neuromasts and *R. paresi* by possessing a reticulate colour pattern on the dorsal fin and the basal portion of the anal fin in males (COSTA, 2007a). With regard to the pattern of reddish dots on body flanks and anal fin *R. punctatus* is similar to *R. rossoi* COSTA, 2006 from rio Paraná drainage and to *R. zygonectes* MYERS, 1927 distributed in río Araguaia, río Tocantins and río Xingu basins (see COSTA, 1995). However, *R. punctatus* clearly differs from *R. rossoi* by a higher number of gill rakers on first gill arch (1+8

Tab. 2. Morphometric data in percents of SL of *Rivulus punctatus* from Paraguay (males = 10, females = 10), results of pair-wise *t*-tests (males vs. females) and coefficients of loading of a PCA of morphometrics. Mean = arithmetic mean, dev = standard deviation, min = lowest value, max = highest value, p = results of t-test, p' = corrected p-value, PC1 = first principal component axis, PC2 = second principal component axis.

Character	**males**				**females**				**t - test**		**coefficients**	
	mean	dev	min	max	mean	dev	min	max	p	p'	PC1	PC2
Standard length [mm]	29.4	3.90	24.0	35.2	27.6	2.94	22.1	30.9	0.2700	0.766		
Body depth	20.1	1.33	18.6	22.0	19.5	1.49	16.2	21.0	0.3540	0.766	-0.165	0.171
Caudal peduncle depth	13.3	0.89	11.7	14.3	12.6	0.62	12.1	13.9	0.0738	0.527	-0.224	-0.017
Predorsal length	77.4	2.18	74.6	80.9	78.9	1.23	77.2	81.4	0.0753	0.527	0.006	-0.081
Prepelvic length	49.6	1.46	47.1	51.9	51.1	1.60	49.2	54.0	0.0413	0.372	0.009	-0.158
Preanal length	61.7	0.87	60.4	63.1	64.0	1.47	61.7	66.4	0.0005	0.006	0.057	-0.124
Dorsal-fin base length	9.8	1.54	7.2	12.2	9.5	0.90	8.5	11.3	0.6295	0.766	-0.435	-0.701
Anal-fin base length	21.5	0.91	20.0	22.7	20.1	1.52	18.5	22.6	0.0187	0.187	-0.194	-0.023
Totallength	129.9	4.85	123.8	135.6	130.4	2.93	125.4	134.7	0.7656	0.766	-0.078	-0.095
Pelvic-fin length	12.4	1.48	10.1	15.2	10.0	1.50	7.5	11.8	0.0023	0.025	-0.783	0.497
Headlength	25.2	1.62	22.9	28.6	26.1	1.14	24.4	28.1	0.2030	0.766	-0.064	-0.234
Eye diameter	8.0	0.79	7.0	9.1	8.2	0.74	7.5	9.9	0.4498	0.766	-0.263	-0.347

versus 1+6 in *R. rossoi*) and the lack of orange stripes on the caudal fin of males. From *R. zygonectes* it is distinguished by a lower number of dorsal-fin rays (8–9 in *R. punctatus* versus 9–11 in *R. zygonectes*), less scales in longitudinal series (30–32 mode 31 in *R. punctatus* versus 32–38 in *R. zygonectes*) and elongated caudal fin (versus caudal fin more or less rounded and not particularly elongated in *R. zygonectes*).

Because of the variation in caudal-fin and body flank patterns in *R. punctatus* (see description above, Collier & Steinberg, 1977; Huber, 1992; Staeck & Schindler, 1994; Seegers, 2000) the possibility appears not unlikely that more than one species is included in the current concept of this taxon. However, the facts that there is no determined correlation neither between morphometrics and geographic position ($r=-0.388$, $p>0.05$, fig 15 for scatter plot) nor between caudal-fin pattern and locality ($r=0.032$, $p>0.1$, see fig. 16), make us regard all the examined populations of *R. punctatus* as members of a single evolutionary entity in the sense of the ESC (Wiley, 1978). This view is additionally supported both by the observation of a change of caudal fin pattern during ontogenesis in at least some specimens (Staeck & Schindler, 1994) and the high variability of colour patterns and morphological data reported for closely related species (Huber, 1992, 2007; Costa, 1995).

Further the lack of any clear physiogeographical barriers in the lowlands apparently prevent the isolation of populations and make it more likely that only one species is involved. Consequently it can be assumed that *R. punctatus* inhabits the floodplains of the río Paraguay and the río Paraná lowlands in Brazil, Bolivia, Paraguay and Argentina (Etzel, 1985, 1991; Huber, 1992; Staeck & Schindler, 1994; Costa, 1995, 2003a).

The habitats preferred by *R. punctatus* are characterised by a low depth (usually less than 30 cm) of water, slow or no currents and muddy or sandy (<3 mm diameter) substrates. *Rivulus punctatus* usually inhabits the zone close to the surface of the water between marsh and water plants or submerged bushes and grass. The analysis of the food composition of *R. punctatus* shows that major components are allochtone insects (Staeck & Schindler, 1994) which probably correlates with the preferred habitats (shallow water close to the surface). The biotopes of *R. punctatus* documented here are generally similar to those described for other species of *Melanorivulus* (e. g. Bastos & Lourenço, 1983 for *R. pictus* [misidentified as *R. punctatus*] and Costa, 2003b, 2003c, 2005, 2007b for further species) and seem to be characteristic of all the members of this group.

The documented variability for *R. punctatus* raises the question about the species limits within the subgenus. Acknowledging the variability in chromatic character states Costa (1995) synonymized the taxa *R. apiamici* Costa, 1989, *R. pinima* Costa, 1989, and *R. vittatus* Costa, 1989, with *R. pictus* Costa, 1989. But in 2005 Costa revalidated all three species and described additional similar taxa without a comprehensive discussion of species limits and taxonomy. Therefore, the diagnoses of all these species may be considered as provisional hypotheses until a comprehensive phylogeographic analysis of *Melanorivulus* is available. At the moment it is difficult to obtain such one, because

every year new populations of endemic evolutionary units are discovered in the upper drainages of the river systems (see COSTA, 2005, 2006b, 2007a, 2007b).

Acknowledgements

We thank GERHARD HESSFELD (Hamm) and FRANZ TERSTIEGE (J.A. SALDIVAR) for their diverse helps and the Secretaria del Ambiente, Ministerio de agricultura y ganaderia, oficina central de pesca in Asuncion for the grant permission to catch fishes for scientific purpose. Thanks also to ERWIN SCHRAML (Augsburg) for reading earlier stages of the manuscript, to AXEL ZARSKE (MTD) for the loan of the specimens and to WOLFGANG STAECK (Berlin) for his comments and helpful suggestions, which significantly improve the manuscript.

References

BASTOS, E.K. & LOURENÇO, W.R. (1983): L'habitat et biotope de *Rivulus punctatus* Boulenger, 1895, dans la region du Distrct Fédéral, Brasil (Pisces, Cyprinodontidae). – Revue française de Aquariologie, **10**(1): 1–4.

BLACKITH, R.E. & REYMENT, R.A. (1971): Multivariate morphometrics. – Academic Press, London, 412 pp.

BOULENGER, G.A. (1895): Viaggio del dottor Alfredo Borelli nella Repubblica Argentina e nel Paraguay. XII. Poissons. – Boll. Mus. Zool. Anat. Comp. Torino, **10**(196): 1–3.

COLLIER, G.E. & STEINBERG, L. (1977): Identification, care and breeding of *Rivulus punctatus* (NSC-11). – Journ. Americ. Killifish Assoc., **7**(10): 349–355.

COSTA, W.J.E.M. (1988): 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. (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.

COSTA, W.J.E.M. (1995): Revision of the *Rivulus punctatus* species-complex (Cyprinodotiformes: Rivulidae). – Ichthyological Exploration of Freshwaters, **6**: 207–226.

COSTA, W.J.E.M. (2003a): Family Rivulidae (South American annual fishes): 526–548. – In: REIS, R.E., KULLANDER, S.O. & FERRARIS, C.J. Jr. (Eds.). Check list of the freshwater fishes of South and Central America. Porto Alegre, Edipucrs, 729 pp.

COSTA, W.J.E.M. (2003b): *Rivulus paracatuensis* n. sp. (Cyprinodontiformes: Rivulidae): a new rivuline species from the rio São Francisco basin, Brazil. – Aqua, Journal of Ichthyology and Aquatic Biology, **7**: 39–43.

COSTA, W.J.E.M. (2003c): A new species of the genus *Rivulus* Poey, 1860 from the Parnaiba river basin, northeastern Brazil (Teleostei, Cyprinodontiformes, Rivulidae). – Boletim do Museu Nacional, Rio de Janeiro, **511**: 1–7.

COSTA, W.J.E.M. (2004): Relationships and redescription of *Fundulus brasiliensis* (Cyprinodontiformes: Rivulidae), with description of a new genus and notes on the classification of the Aplocheiloidei. – Ichthyological Exploration of Freshwaters, **15**(2): 105–120.

COSTA, W.J.E.M. (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**(1): 69–82.

COSTA, W.J.E.M. (2006a): Relationships and taxonomy of the killifish genus *Rivulus* (Cyprinodontiformes: Aplocheiloidei: Rivulidae) from the Brazilian Amazonas river basin, with notes on historical ecology. – Aqua, Journal of Ichthyology and Aquatic Biology, **11**(4): 133–175.

COSTA, W.J.E.M. (2006b): *Rivulus kayapo* n. sp. (Teleostei: Cyprinodontiformes: Rivulidae): a new killifish from the serra dos Caiapós, upper rio Araguaia basin, Brazil. – Zootaxa, **1368**: 49–56.

COSTA, W.J.E.M. (2007a): *Rivulus bororo* and *R. paresi*, two new killifishes from the upper Paraguay River basin, Brazil (Teleostei: Rivulidae). – Ichthyological Exploration of Freshwaters, **18**(4): 351–357.

COSTA, W.J.E.M. (2007b): Five new species of the aplocheiloid killifish genus *Rivulus*, subgenus *Melanorivulus*, from the middle Araguaia River basin, central Brazil (Teleostei: Cyprinodontiformes: Rivulidae). – Aqua, International Journal of Ichthyology, **13** (3): 55–68.

ETZEL, V. (1985): Zwei weitere Fundorte von *Rivulus punctatus* in Paraguay. – DKG-Journal, **17**(8): 122–128.

ETZEL, V. (1991): Zur Verbreitung von *Rivulus punctatus* Boulenger, 1895. – DKG-Journal, **23**(1): 8–13.

HAMMER, Ø., HARPER, D.A.T. & RYAN, P.D. (2004): Past: Paleontological statistics software package for education and data analysis. – Paleontologia electronica, **4**: 9.

HAMMER, Ø. & HARPER, D.A.T. (2006): Paleontological data analysis. – Blackwell Publishingm Malden. 344pp.

HAMMER, Ø., HARPER, D.A.T. & RYAN, P.D. (2007): PAST – Palaeontological Statistics. Version 1.71 (computer programme).

HENN, A. W. (1916): On various South American poeciliid fishes. – Annals of the Carnegie Museum, **10**: 93–142.

HUBER, J.H. (1992): Review *of Rivulus:* ecobiogeography - relationships. – Paris, Société Française d'Ichtyologie. 572 pp.

HUBER, J.H. (1995): Nouvelles collections de cyprinodontes paraguayens, avec description de 4 espèces rivulines inedites et redecourvete d'une espèce a la localite typique jusquálors indeterminee. – Killi-Contact, **23**: 1–24.

HUBER, J.H. (1999): Updates to the phylogeny and systematics of the neotropical Cyprinodont genus *Rivulus* and its allied (Cyprininodontiformes: Rivulidae). – Cybium, **23**(1): 29–52.

HUBER, J.H. (2007): Killi-Data online. http://www.killi-data.org/ [access 10.VIII 2007]

LASSO-ALCALÁ, O.M., TAPHORN D.C., LASSO, C.A. & LEÓN-MATA, O. (2007): *Rivulus sape*, a new species of killifish (Cyprinodontiformes: Rivulidae) from the Paragua Riversystem, Caroní River drainage, Guyana Shield, Venezuela. – Zootaxa, **1275**: 21–29.

LOZAN, J. L. (1992): Angewandte Statistik für Naturwissenschaftler. – Pareys Studientexte **74**. Verlag Paul Parey, Berlin/Hamburg. 237 pp.

Regan, C. T. (1912): A revision of the poeciliid fishes of the genera *Rivulus*, *Pterolebias* and *Cynolebias*. – Annals and Magazine of Natural History (Ser. 8), **10**(59): 494–508.

Schindler, I. (2003): BONFERRONI-p – a computer program for the Bonferroni corrections. – IS-Online-Publication, No. 4.

Seegers, L. (2000): Killifishes of the World – New World Killis. Aqualog, Verlag A.C.S., Mörfelden-Walldorf, 224pp.

Staeck, W. & Schindler, I. (1994): Killifische aus dem Flußsystem des Rio Paraguay: 3. *Rivulus punctatus*. – Aquararien- Terrararien Zeitschrift, **47**(6): 374–380.

Thomerson, J.E., Nico, L. & Taphorn, D.C. (1992): *Rivulus tecminae*, a new killifish from Amazonas Territory, Venezuela (Cyprinodontiformes: Rivulidae). – Ichthyological Exploration of Freshwaters, **2**(4): 289–296.

van Velzen, J., Bouton, N. & Zandee, R. (1992): A procedure to extract phylogenetic information from morphometric data. – Netherlands Journal of Zoology, **48**: 305–322.

Wiley, E. (1978): The evolutionary species concept reconsidered. – Systematic Zoolology, **27**: 17–26.

Woodman, N. (2000): *Cryptotis merriami* Choate in Costa Rica: Syntopy with *Cryptotis nigrescens* (Allen) and possible character displacement (Mammalia: Insectivora). – Caribbean Journal of Science, **36**(3/4): 289–299.

Yoshioka, K. (1997–2000): KyPlot. Version 2.0. Computer programme.