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Rivulus mahdiaensis, a new killifish from central Guyana (Cyprinodontiformes: Rivulidae)

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Abstract

Rivulus mahdiaensis, new species, was found in a small creek, a tributary of the Potaro River, near Mahdia, central Guyana. Males differ from all other species of the genus *Rivulus* by possessing elongate anal and dorsal fins, and an extremely large and lyre-shaped caudal fin with long extensions. Females differ from other species of the genus, except for three closely related species, by the presence of a narrow, dark longitudinal band on the sides. *R. mahdiaensis* possesses a unique karyotype (2n=38) among members of this group. Comparison of mitochondrial DNA sequences place this species as a member of a monophyletic clade which includes other small species from the Guyana shield and adjacent areas of northeastern South America.

Resumen

Rivulus mahdiaensis, una nueva especie, se encontró en un pequeño arroyo tributario del Rio Potaro cercano a la localidad de Madhia , en la Guyana Central. Los machos difieren de todas las otras especies del género Rivulus por sus aletas anales y dorsales extremadamente largas y una aleta caudal en forma de lira con largos filamentos en sus extremos. Las hembras difieren de otras especies del Género , excepto por tres especies relacionadas, por la presencia de una estrecha y obscura banda longitudinal en ambos lados. *R. mahdiaensis* posee un único cariotipo (2n=38) entre los miembros de este grupo. Comparando la secuencia de DNA mitocondrial podemos situar a esta especie como un miembro de un clado monofilético que incluye otras pequeñas especies de la región de la Guyana y áreas vecinas del Noroeste de Sudamérica

Key words: Cyprinodontiformes, Rivulidae, *Rivulus*, Guyana, molecular phylogeny, karyotype, new species

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Introduction

Since Eigenmann's expedition of 1908 (Eigenmann 1909) there have been very few recorded collections of *Rivulus* Poey, 1860 from Guyana. [Lang in 1922, Pinkus in 1933 and Cichocki in 1971 (Huber,1992); Thomerson in 1975 (pers. comm.), de Rham in 1976 (Vermeulen & Isbrücker, 2000) and Hrbek in 1999 (pers. comm.)]. Therefore, knowledge of the diversity and distribution of Guyanan *Rivulus* is very limited. The senior author visited Guyana six times from 1991 to 2004 in order to study and document the cyprinodont fauna, particularly the genus *Rivulus*, of this country. A secondary goal was to recollect the *Rivulus* species described by Eigenmann (1909). During the collecting trip of 1991, W. Suijker, F. Vermeulen and their wives were able to find and collect *Rivulus breviceps* Eigenmann, *Rivulus frenatus* Eigenmann, *Rivulus stagnatus* Eigenmann and *Rivulus waimacui* Eigenmann. *Rivulus agilae* Hoedeman was also collected at several sites. During the collecting trip of 1995 W. Suijker and his wife Y. Suijker-Jansen discovered a species new to science near the village Mahdia in central Guyana.

Material and methods

The color descriptions are based on color slides and observations of three wild caught fish under aquarium conditions. These three specimens and an additional six specimens preserved in the field were used for meristic and morphometric measurements. The holotype, allotype and four paratypes have been deposited in the Zoological Museum Amsterdam (ZMA) and three additional paratypes have been deposited with the Field Museum of Natural History (FMNH). Three paratypes provisionally deposited with the Zoological Museum Amsterdam will be deposited with the University of Guyana pending completion of arrangements for transfer.

Meristics and morphometrics

Measurements and counts follow Huber (1992). All visible rays from the anal and dorsal fins were counted. The measurements were taken with vernier calipers to the nearest tenth of a millimeter and are presented in percentages of the standard length

Karyotype

Metaphase preparations of chromosomes were prepared from gill epithelium of a single wild-caught male as described by Kligerman and Bloom (1977).

Molecular analysis

Isolation of DNA and amplification of portions of the mitochondrial genes for cytochrome b, 12S RNA and 16S RNA was done as described by Murphy and Collier (1996 and 1997) and Murphy *et al.* (1999). All sequences except those for *R. mahdiaensis*

were previous published (Murphy and Collier, 1996; Murphy *et al.* 1999). GenBank accession numbers for the taxa used are: *R. geayi* (AF002433, AF002537, AF002483), *R. strigatus* (AF002434, AF002538, AF002484), *R. agilae* (AF002432, AF002536, AF002482), *R. frenatus* (AF002435, AF002539, AF002485), *R. xiphidius* (AF002436, AF002540, AF002486), *R. lyricauda* (AF002439, AF002543, AF2489), *R. rectocaudatus* (AF002440, AF002544, AF002490), *R*. sp. Tobogan (AF002437, AF002541, AF002487), *R.* sp. DCT89-132 (AF002438, AF002542, AF002488), *R. cylindraceus* (U41799, AF002533, U41781) and *R. roloffi* (U41798, AF002434, U41780). The sequences for *R. mahdianensis* (GenBank accession numbers DQ501248, DQ501249, DQ501250) were aligned manually with the previous established alignment. Neighbor joining analysis (Saitou and Nei 1987) was done in MEGA 2.1 (Kumar et al., 2001) using the Kimura 2-parameter model of substitutions. Maximum parsimony analyses (MP) were performed using 100 random addition replicates and TBR branch swapping. Starting trees were obtained by neighbor joining for the maximum likelihood search (ML). Non parametric bootstrap analyses were performed using 100 heuristic replicates with TBR branch swapping and random taxon additions. MP and ML analyses were performed in PAUP*4.0b10 (Swofford, 2002). Settings for the model of DNA sequence evolution were estimated using the Akaiki Information Criterion implemented in Modeltest (Posada and Crandall, 1998).

***Rivulus mahdiaensis,* new species**
(Figure 1)

Holotype. ZMA 123.720 (**Allotype**, ZMA 123.721; **Paratype**, ZMA 123.722) Guyana, Mazaruni-Potaro District, Blackwater Creek, tributary of the Potaro River, on 5 miles from Mahdia crossing the road from Mahdia to Garroway Stream, 5° 21’ 11” N and 59 ° 08’48” W. Field code Guy 95/11. Coll W. Suijker & Y. Suijker-Jansen, 1995 April 12 at 14.00 hours.

3 **Paratypes**. FMNH 113582, FMNH113583 and FMNH113584. Guyana, Mazaruni-Potaro District, Blackwater Creek, tributary of the Potaro River, on 5 miles from Mahdia crossing the road from Mahdia to Garroway Stream, 5° 21’ 11” N and 59 ° 08’48” W. Field code Guy 95/11. Coll W. Suijker & Y. Suijker-Jansen, 1995 April 12 at 14.00 hours.

3 Paratypes. ZMA 123.853, ZMA 123.854 and ZMA 123.855 Guyana, Mazaruni-Potaro District, Blackwater Creek, tributary of the Potaro River, on 5 miles from Mahdia crossing the road from Mahdia to Garroway Stream, 5° 21’ 11” N and 59 ° 08’48” W. Field code Guy 95/11. Coll W. Suijker & Y. Suijker-Jansen, 1995 April 12 at 14.00 hours.

Diagnosis

Males of *Rivulus mahdiaensis* differ from all other species of the genus *Rivulus* by their unique long and lyre-shaped caudal fin with filaments and by the elongated unpaired fins.

Females show a narrow dark lateral band and share this distinctive characteristic only

with *Rivulus frenatus*, *R. xiphidius* and perhaps *R. campelloi* (Huber 1992). Females of *Rivulus mahdiaensis* have an ocellus on the dorsal part of the caudal peduncle. This is unusual because the small species of the Guyana plateau have no ocellus on the caudal peduncle. This ocellus is often present in females of many species of *Rivulus* and hence is often called a "Rivulus spot". The meristics (D 6–7, A 8–9 and D/A 4–5) (Table 1) are lower than those of the other small species on the Guyana plateau (Table 2).

Comparison of mitochondrial DNA sequences place this species as a member of a monophyletic clade which includes *R. agilae*, *geayi* Vaillant, *frenatus*, *xiphidius* Huber and *lyricauda* Thomerson, Berkenkamp & Taphorn. *R. mahdiaensis* possesses a unique karyotype (2n=38) among members of this group.

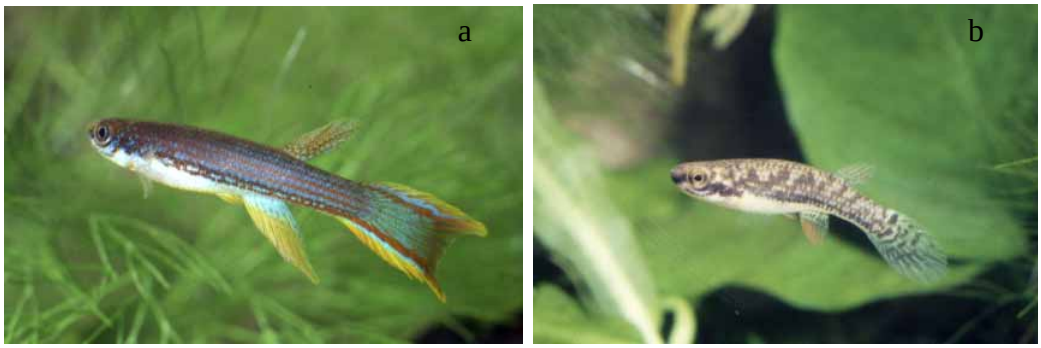

FIGURE 1. *Rivulus mahdiaensis*, new species, a. male; b. female.

Description

This new species is small and slender and has a uniquely shaped caudal fin. All rays of the caudal are proportionately longer than for most *Rivulus* while the dorsal and ventral rays are extended to an even greater degree in mature males. Thus, it is the first record of a *Rivulus* species with an exaggerated lyre tail. The dorsal and anal fin rays are also uniquely elongated. The longest anal fin rays of mature males are at least 150% of the body depth at anal fin origin, whereas in all other known species of the genus they are less than 100% of the body depth.

Coloration

Live colors. Male: The sides are blue reflective and show five or six continuous lines. The three upper lines are red. The lower lip is black and a wide stripe runs via the eyes to the gill covers where it splits into two lines and ends at anal fin height. These two lower lines are more dark red to black on the sides before they merge to one red band posterior to the anal fin and continue through the mid-lower portion of the caudal fin. The lyre shaped caudal fin has the following color pattern. There is a yellow stripe along the dorsal margin which continues to the end of the dorsal extension, followed interiorly by a blue stripe and then a red one. The ventral margin of the caudal is also yellow which continues to the end of the ventral extension, followed interiorly by a blue stripe and then a red one. The middle

TABLE 1. Meristics and morphometrics for the holotype and paratypes of Rivulus mahdiaensis.

	Holotype	Paratypes				
	Males					
Collection-item	ZMA	FMNH	FMNH	FMNH	ZMA	ZMA
	123.720	113582	113583	113584	123.853	123.854
1. Merestic data	mm	mm	mm	mm	mm	mm
Total length	33.4	31.0	39.0	32.2	34.0	28.1
Standard length	24.1	24.6	30.5	27.4	28.2	22.2
Greatest depth of body	5.2	5.9	6.5	6.2	5.9	5.0
Length of head	6.0	5.8	6.5	6.0	6.1	6.4
Eye diameter	2.1	2.0	1.8	2.0	1.8	1.9
Width of interorbital space	4.2	4.0	4.5	5.0	4.2	3.5
Lenght of snout	1.2	1.5	1.5	1.2	1.5	1.5
Depth of caudal penduncle	3.3	3.6	4.5	3.0	4.2	3.1
Pre-dorsal length	17.4	17.5	21.8	19.0	21.0	16.8
Pre-anal length	15.0	15.0	20.0	17.5	19.6	14.3
Pre-ventral length	12.0	13.0	17.0	14.9	16.0	12.0
2. Meristic data fins						
Number of dorsal fin rays	7	6	7	7	6	6
Number of anal fin rays	9	8	8	9	8	8
Dorsal insertion to anal insertion	+5	+5	+4	+4	+4	+4
(D/V)						
3. Scalation						
Number scales LL + C	31	30	31	31	30	30
Transverse scales (d-a)	9	8	9	9	9	9
4. Body ratios						
Body height to TL	6.4	5.3	6.0	5.2	5.8	5.6
Body height to SL	4.6	4.2	4.7	4.4	4.8	4.4
Length of head to TL	5.6	5.3	6.0	5.4	5.6	4.4
Length of head to SL	4.0	4.2	4.7	4.6	4.6	3.5
Eye diameter to length of head	2.9	2.9	3.6	3.0	3.4	3.4
Eye diameter to length of snout	0.6	0.8	0.8	0.6	0.8	0.8
Eye diam.to interorbital space	2.0	2.0	2.5	2.5	2.3	1.8

to be continued.

TABLE 1 (continued).

Collection-item	Paratypes Females ZMA 123.855	ZMA 123.721	ZMA 123.722	Average	
1. Merestic data	mm	mm	mm	X	SD
Total length	30.0	31.8	35.0	32.7	3.2
Standard length	28.3	25.0	27.0	26.4	2.6
Greatest depth of body	5.1	7.0	6.3	5.9	0.7
Length of head	5.8	6.0	6.5	6.1	0.3
Eye diameter	1.9	2.0	2.2	2.0	0.1
Width of interorbital space	4.2	4.2	4.8	4.3	0.4
Lenght of snout	1.3	1.5	1.6	1.4	0.2
Depth of caudal penduncle	3.2	3.5	4.0	3.6	0.5
Pre-dorsal length	21.0	18.0	18.6	19.0	1.8
Pre-anal length	19.5	16.0	16.1	17.0	2.2
Pre-ventral length	15.4	13.5	13.5	14.1	1.8
2. Meristic data fins					
Number of dorsal fin rays	7	6	7	6.6	0.5
Number of anal fin rays	9	8	9	8.4	0.5
Dorsal insertion to anal insertion (D/V)	+5	+5	+5	4.6	0.5
3. Scalation					
Number scales LL + C	31	30	30	30.4	0.5
Transverse scales (d-a)	8	8	9	8.6	0.5
4. Body ratios					
Body height to TL	5.9	4.5	5.6	5.6	0.5
Body height to SL	4.9	3.6	4.3	4.4	0.4
Length of head to TL	5.9	5.3	5.4	5.4	0.5
Length of head to SL	4.9	4.2	4.2	4.3	0.4
Eye diameter to length of head	3.1	3.0	3.0	3.1	0.3
Eye diameter to length of snout	0.7	0.8	0.7	0.7	0.1
Eye diam.to interorbital space	2.2	2.1	2.2	2.2	0.2

part of the caudal is greenish. The elongated dorsal fin is reflective blue with a many red spots. The elongated anal fin is yellow distally and blue basally with a few red spots. The

ventral fins are yellow with some red spots. The pectoral fins are clear. The dorsum is brown and the belly is dirty white.

Female: dorsum and sides are light brown with irregular dark spots that form a pattern of diagonal bands. There is a black band on the lower lip, which runs, as in the males, via the eye to the lower part of the base of the caudal fin. Above this band there are two lines of indistinct red spots. The caudal fin is clear and shows irregular black markings that form three vertical-lines. On each fin ray between the vertical-lines is a light yellow spot. Females also have an ocellus on the dorsal part of the caudal peduncle. This ocellus is often present in females of many species of *Rivulus* and hence is often called a "Rivulus spot" . The proximal 1/3 of the anal fin is light blue with two rows of dark spots. The distal 2/3 is dark red. The dorsal fin is clear with irregular red spots. The ventral and pectoral fins are clear.

Color in alcohol. Males: Sides are light brown, on the lower part there are two or three rows of dark red spots, no longitudinal band is visible. All fins are clear without spots. Females are light brown with darker brown spots with no visible longitudinal stripe. All fins are clear, except the caudal fin, which has the same pattern as in life.

The holotype and allotype have a D type pattern of frontal scalation (*sensu* Hoedeman 1958). The other specimens were not examined for this character as it is no longer used in diagnostic keys.

Etymology

Rivulus mahdiaensis is named after the village Mahdia near where it was discovered.

Distribution and habitat

The type locality of *Rivulus mahdiaensis* is a small black water creek, called Blackwater Creek, near the village of Mahdia. This creek is a tributary of the Potaro River, 4 miles above Kangaruma landing, on the right bank of the Potaro River, Mazaruni-Potaro District, Guyana. The location is about 250 meters above sea level.

Rivulus mahdiaensis was collected in the shallow parts of this black water forest-creek between the roots of plants growing at the bank of the creek (Fig. 2). We also caught them in little pools along the banks of the creek. They prefer the dark parts of the biotope. *Rivulus waimacui*, *Apistogramma ortmanni*, *Nannacara bimaculata*, *Poecilocharax bovalli* were caught in the same creek. The temperature of the water was 25° C. at 14.00 hours. The pH was 4.5 and the Total Dissolved Solids was < 1DH.

A second location of this new species was found in 1997 along the road between Bartica and Garraway Stream by the author and his wife Y. Suijker-Jansen, F. Vermeulen and M. Vermeulen. This location is another blackwater creek, north of the Potaro River, which crosses the road from Bartica to Mahdia at 77 miles from Bartica (Fig. 3). No specimens were preserved from this second location. This species may have a very limited distribution or be uncommon where it is found as no other collections of *R. mahdiaensis*

were made at the eight different sites around Mahdia and along the road from Bartica to Mahdia visited during collecting trips of 1991, 1995, and 1997.

Aquarium behavior

Rivulus mahdiaensis is a peaceful species, which can be kept in a group of several pairs. Sometimes there is some aggression or competition between the males but they do not appear to damage each other. They do not like bright light. The pH of the water must be around 6 and temperature around 23° C. Under aquarium conditions they lay their eggs (~2 mm in diameter) in plants with fine leafs, like *Vesicularia dubyana* (Java moss). They will also spawn in artificial spawning mops made of yarn depositing eggs in the top and at the bottom. The eggs develop in 18 to 21 days. The newly hatched fry are immediately able to take *Artemia* nauplii. Young fish have the plainer pattern of females until ten to twelve weeks of age when the color pattern of males begins to appear.

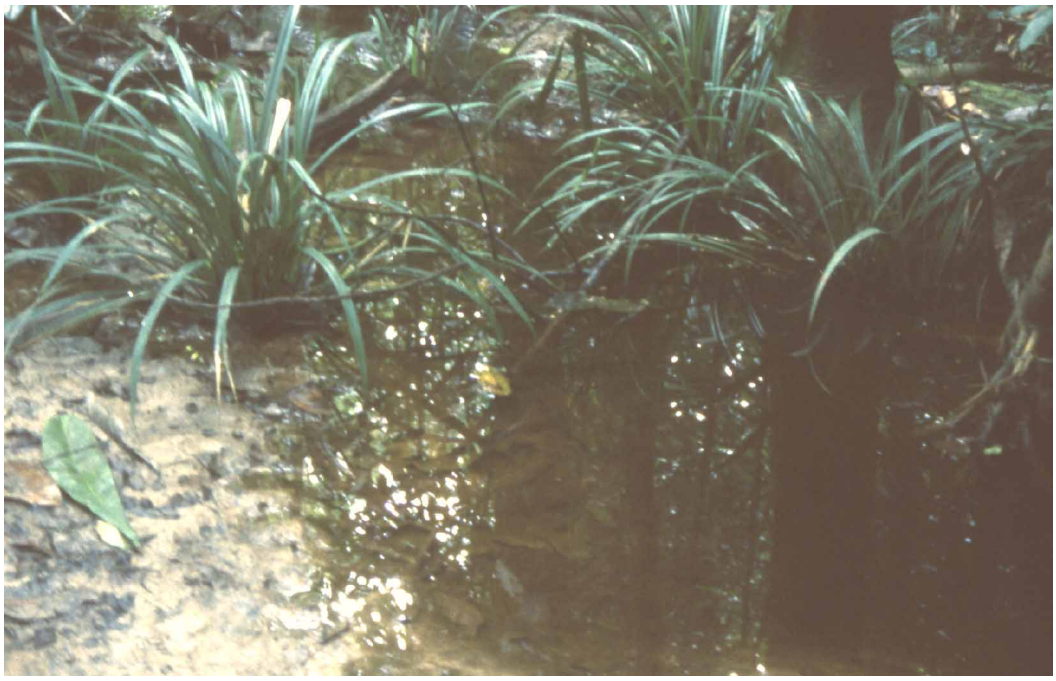

FIGURE 2. Biotope of *Rivulus mahdiaensis*. Small blackwater stream near the village of Mahdia from which the holotypes were collected.

Discussion

One of the hallmarks of the genus *Rivulus* is morphometric homogeneity (Huber, 1992). However, *R. mahdiaensis* is easily distinguished from related small species of *Rivulus* by the following criteria. It differs from *Rivulus breviceps*, *Rivulus lyricauda*, *Rivulus agilae*, *Rivulus torenticola* Vermeulen & Isbrücker, *Rivulus gransabanae* Lasso, Taphorn &

Thomerson, *Rivulus frenatus* and *Rivulus xiphidius* by its lower number of dorsal and anal fin rays as well as a more anterior insertion of the dorsal fin relative to the anal fin (D/A metric of Huber, 1992). The low number of lateral line scales (30) in *R. mahdiaensis* is similar to that of *R. lyricauda* (30.6) and *R. xiphidius* while this number averages greater than 31 for the other species listed above (Huber 1992) (Table 2). Females of *R. mahdiaensis* have a caudal ocellus as do *R. agilae* and *R. geayi*. However, it is lacking in females of *R. lyricauda*, *R. frenatus*, *R. xiphidius*, *R. gransabanae*, and *R. torenticola*. *R. mahdiaensis* also differs from all of these species by body shape, shape of anal and dorsal fin and shape and color pattern of the caudal fin.

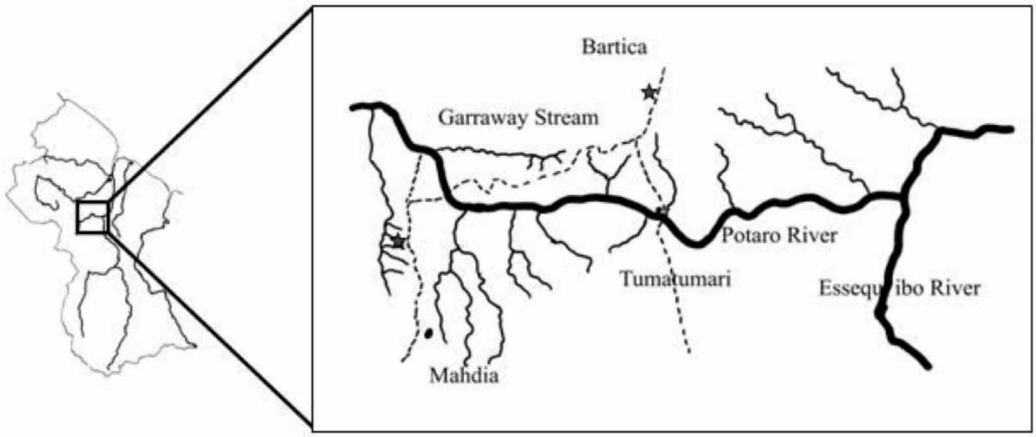

FIGURE 3. Map of Guyana. The outline map of Guyana indicates the general area that is shown in greater detail in the insert. Sites at which the senior author collected *Rivulus mahdiaensis* are each marked with a star in the insert map. Precise location of the type locality is given in the text.

R. mahdiaensis represents a unique and distinct phenotype within the genus *Rivulus*. The color pattern, especially that of the female, suggested a relationship with *R. frenatus* and *R. xiphidius*, as females of these species also are characterized by a broad, dark longitudinal stripe on the sides. The broad dark longitudinal stripe also appears in *R. lyricauda* when it is stressed (Thomerson *et al.* 1991). Although *R. lyricauda* has modest extensions to the dorsal and ventral rays of the caudal, these extensions are not as long as those of *R. mahdiaensis* nor are the rays of all unpaired fins as elongated. All four of these species are also characterized by having very dark to black lips.

The molecular phylogeny of *R. mahdiaensis* and some of its related species reveals considerable congruence with the morphological features discussed above (Fig. 4). The closest relative among the species sampled is *R. lyricauda*, the only other species of *Rivulus* with a clear tendency to form a lyre shaped caudal fin. These two sister taxa are members of a monophyletic group that also includes *R. frenatus*, *R. xiphidius*, *R. agilae*, *R. geayi*, and *R. strigatus*. [We are aware that *R. strigatus* Regan has been synonymized with *R. geayi* (Huber 1992), however the two geographically distinct populations used for the

DNA sequencing (*Guyana* and Belem, Brazil) displayed significant sequence differences and we retained the name *strigatus* in Fig. 4]

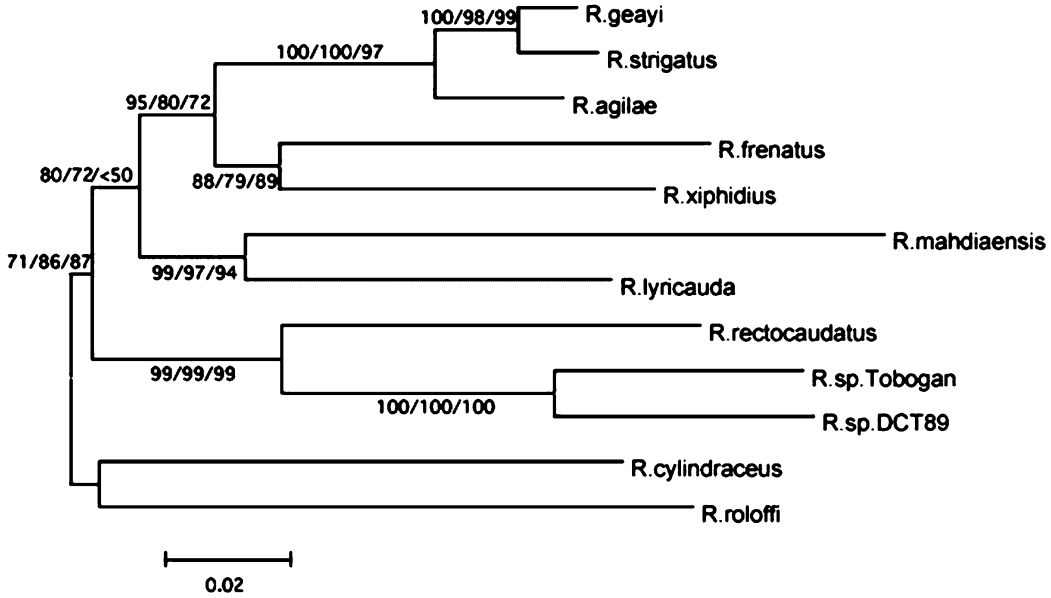

FIGURE 4. Phylogenetic relationships of *Rivulus mahdiaensis* and its near relatives. This neighbor-joining (NJ) tree is identical in topology to the best MP and ML (GTR+gamma+I; -lnL=5825.2334, α=0.6778, pinvar=0.4051) trees. The numbers subtending the nodes are bootstrap values for NJ, MP and ML, respectively.

TABLE 2. Meristic data for *Rivulus mahdiaensis* and near relatives. Data for *R. mahdiaensis* are from Table 1. Data for *R. torenticola* are from Vermeulen and Isbrücker, 2000. Remaining data are from Huber, 1992.

	Dorsal fin rays	Anal fin rays	Insertion of dorsal fin relative to anal fin	Lateral line scales
R. mahdiaensis	6–7	8–9	4–5	30–31+2
R. agilae	8–9	11–12	5–7	33+2
R. breviceps	9–10	12	6–7	35
R. frenatus	7	12–13	7–8	40
R. geayi	9	9–12	6–7	35
R. gransabanae	7–11	9–13	7	30–34+3
R. lyricauda	8–10	11–14	6	29–32
R. torenticola	8	10–11	9	31–32+2
R. xiphidius	9	11–12	7–8	30–32+1

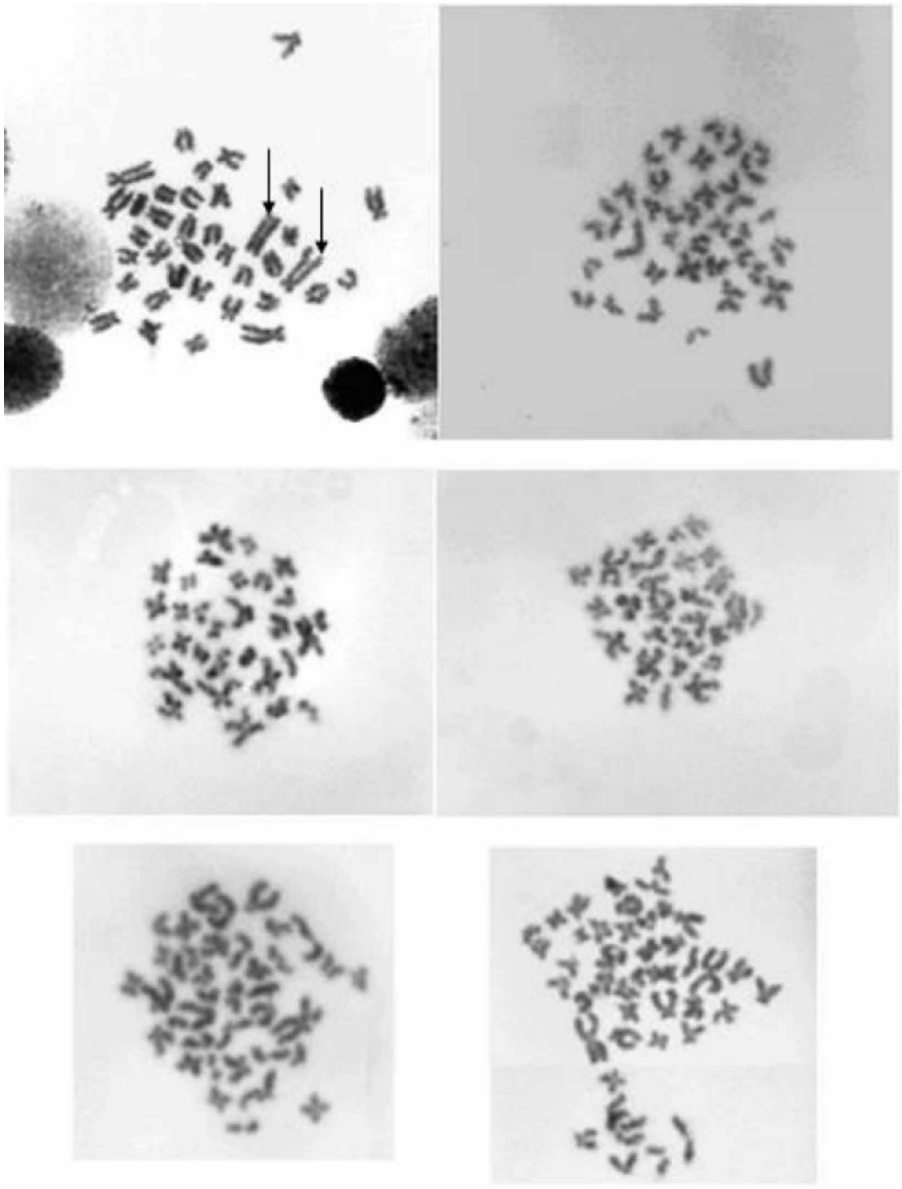

FIGURE 5. Karyotypes of *R. mahdiaensis* and related species: a. *R. mahdiaensis* (2n=38) type locality; b. *R. lyricauda* (2n=40) Canaima, Venezuela*;* c. *R. xiphidius*, (2n=30) 15km south of Kourou, Guyana; d. *R. frenatus* (2n=30) 40 Miles Creek, Guyana; e. *R. strigatus*, (2n=42) Belem, Brazil; f. *R. geayi* (2n=44) Crique Blanche, Guyane. The exceptionally large pair of acrocentric elements unique to *R. mahdiaensis* is marked by arrows (5a).

The ten taxa represented in Figure 4 and rooted in that figure with *R. cylindraceus* and *R. roloffi* when placed in the larger context of the family Rivulidae, represents a monophyletic clade that diverged from other members of the family early in its history (Murphy *et al*., 1999: Hrbek and Larsen, 1999). *Rivulus breviceps,* and *R. gransabanae* are

also members of this group based upon independent sequence data (Hrbek *et al.*, 2004), but were not available for inclusion in this analysis. *Rivulus torenticola* may also be a member of this group.

The reduced chromosome number seen for *R. mahdiaensis* (Fig. 5a) is an unusual karyotype in that *Rivulus* karyotypes rarely show reduction in chromosome number below 2n=44 (Scheel 1972 , Collier, unpublished data). However, within the group of ten taxa shown in Figure 4, most members have reduced chromosome numbers. This reduction is minimal for three species in this clade which have most recently diverged from each other [*R. agilae* is 2n=44 (Zoch *et al*. 1989), *R. geayi* is 2n=42 (Fig. 5f), *R. strigatus* from Belem is 2n=42 (Fig. 5e)], whereas it is more pronounced for *R. mahdiaensis* and the three other related species [*R. mahdiaensis* is 2n=38 (Fig. 5a), *R. lyricauda* is 2n=40 (Fig. 5b), *R. frenatus* and *R. xiphidius* are both 2n=30 (Fig. 5c & d),]. Within this latter group *R. mahdiaensis* is also distinctive in possessing a pair of an exceptionally large acrocentric chromosome not evident in the other karyotypes examined. One of the remaining species, *R. rectocaudatus* (Fels & de Rham, 1982), is reported to also be 2n=38.

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