

## ***Kryptolebias sepia* n. sp. (Actinopterygii: Cyprinodontiformes: Rivulidae), a new killifish from the Tapanahony River drainage in southeast Surinam**

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### **Abstract**

*Kryptolebias sepia* n. sp. is described from small forest tributaries of the Tapanahony and Palumeu Rivers which form part of the Upper Marowijne River system in southeast of Surinam. This species is distinguished from all other *Kryptolebias* spp. and *Rivulus* spp. by strong melanism on the body, its ability to change color pattern rapidly, the lack of strong sexual dimorphism, and the presence of pronounced adult/juvenile dichromatism.

**Key words:** *Rivulus*, *Kryptolebias*, Guyana Shield, mtDNA, speciation, molecular phylogeny, biodiversity

### **Introduction**

The fauna and flora of the Guyana shield is particularly rich. While extensive floristic surveys have been undertaken, relatively little work has been conducted on the fish fauna of this region. Most surveys have been done in Venezuela, and Brazilian surveys have concentrated primarily on the middle Rio Negro drainage. The first and last major survey of Guyana was conducted by Eigenmann in 1909 (Eigenmann 1912), and more recently his route has been retraced by researchers focusing on loricariid catfishes (Hardman *et al.* 2002). A survey of freshwater fishes of French Guiana has also been published (Keith *et al.* 2000a, b). With the exception of a cichlid survey by Kullander and Nijssen (1989), no systematic ichthyological survey has been done in Surinam. Although these studies concentrated on larger species and not small species inhabiting primary forest streams, they still revealed an incredible diversity of larger and small fish species. Small fish species are an especially interesting component of Guyana shield drainages, in particular those of the

Rio Negro basin. The extremely low nutrient and mineral content of these drainages has been hypothesized to favor reproduction at smaller body size, which then results in community assemblages of small fish species (Weitzman & Vari 1988, Winemiller 1990).

As discussed in Hrbek *et al.* (2004), rivuline diversity is easy to miss since it requires collections in habitats that normally are not part of larger systematic surveys. Rivulid habitats are largely non-overlapping even with those of small ostariophysin species, since rivulids often live in single-species communities in extremely shallow headwaters of creeks, or in peripheral leaf litter. However, rivuline diversity is likely to be extensive since these types of habitat should, in theory, facilitate allopatric diversification.

From 17 August 2000 to 28 August 2000 the first author visited Surinam together with S. Sladkowsky to investigate the biodiversity of the genus *Rivulus*. In addition to species already known from Surinam or surrounding countries, they were able to collect a species unknown to science. This species was found in shallow pools next to a creek, not far downstream from the Amerindian village of Palumeu, together with *Rivulus* aff. *urophthalmus* and *Rivulus* aff. *geayi*. The Palumeu site was revisited from 8 July 2003 to 5 August 2003 to gather additional material and information on the ecology and natural distribution of this taxon.

This paper describes this species as a member of the genus *Kryptolebias*, provides a diagnosis and hypothesis of relationships of this taxon to other *Kryptolebias* within the Rivulidae.

## Materials and Methods

All specimens collected in 2000 were preserved in the field. Part of the 2003 material was preserved in the field, while other specimens were maintained live for further study, including molecular analyses. Geographic coordinates were taken with a handheld GPS 310 from Magellan.

**Animal Husbandry**—Groups of one male with four females were maintained in standard 10 gallon aquaria filled less than half way with rainwater (pH 6, 0.5 DH) at an average ambient temperature of 24°C. Fish were fed *ad libitum*, and 50% water change was performed once weekly. Up to 10 eggs per day were laid in acrylic wool mops suspended from a 2 cm<sup>2</sup> Styrofoam piece. Approximately 50% of the spawned eggs were deposited outside the water, on top of the Styrofoam. Eggs were collected daily, and partitioned into three groups. One third was stored in aquarium water in shallow containers with traces of acriflavin dichloride added as fungicide. Second third was stored on wet peat moss, and the remaining third was stored on moist peat moss. Egg survivability and development was compared among these three methods.

**Morphological Methods**—Preserved specimens were measured with Mututoyo digital calipers, and measurements were rounded off to the nearest tenth of a millimeter.

Counts, measurements and color pattern definitions follow Huber (1992). Nomenclature for frontal squamation follows Hoedeman (1958). Morphometric and meristic characters were analyzed in Excel.

**Molecular Methods**—Mitochondrial DNA sequence data were obtained from five individuals representing the three species of *Rivulus* found in Palumeu. Previously published data (Hrbek & Larson 1999, Hrbek *et al.* 2004) in combination with the newly generated sequence data were used in the phylogenetic analysis. The African aplocheiloids *Aphyosemion elberti*, *A. geryi*, *Epiplatys singa*, *Fundulopanchax gardneri* and *Nothobranchius furzeri* were included as out-group taxa, and the South American Rivulidae were treated as in-group taxa. DNA sequence data used in this study consisted of genes encoding a portion of 12S ribosomal RNA, complete sequences of the NADH2 gene, transfer RNAs coding for valine, glutamine, methionine, tryptophan, alanine, asparagine, cysteine and tyrosine, and the light-strand replication origin. Amplification and sequencing primers as well as sequencing strategy are described in Hrbek and Larson (1999) except that sequence data were obtained using fluorescent rather than radioactive labeling. Cycle sequencing protocol followed the manufacturers recommended conditions for Perkin Elmer BigDye v3.1 fluorescent sequencing kit. Sequence data were determined on an MJ Research BaseStation automatic sequencer.

**Phylogenetic analyses**—A total of 1972 characters were included in the analyses. Phylogenetic relationships were estimated using a maximum likelihood algorithm implemented in PAUP\* v4.10b (Swofford 2002) and Bayesian likelihood algorithm implemented in MRBAYES (Huelsenbeck & Ronquist 2001) under the GTR model (Rodriguez *et al.* 1990) of molecular evolution with rate heterogeneity. Rates for variable sites were assumed to follow a gamma distribution with a portion of sites treated as invariable. The GTR +  $\Gamma$  + I model was suggested as the most appropriate by the software MODELTEST (Posada & Crandall 1998). Maximum likelihood topology was estimated by a heuristic search, with 25 random additions and TBR branch swapping. Parameter values were estimated from the data. Due to the size of the data set, robustness of the maximum likelihood phylogenetic hypothesis could not be assessed by bootstrapping. For Bayesian likelihood estimate of phylogenetic relationships, we ran 5 000 000 generations, sampling trees and branch-length every 1 000 generations. We discarded the lower 10% of the trees in the computation of a 50% majority rule consensus tree. Following Huelsenbeck *et al.* (2001), the percent of times a clade occurs among the sampled trees was interpreted as the probability of that clade existing. These posterior probabilities are true probabilities under the assumed model of substitution (Rannala & Yang 1996); thus we considered clades to be significantly supported when Bayesian posterior probabilities were  $\leq 95\%$ . Phylogenetic relationships were also estimated using maximum-parsimony algorithm as implemented in PAUP\* v4.10b (Swofford 2002); all changes were unordered and were given equal weights. Bootstrap resampling (Felsenstein 1985) was applied to assess support for individual nodes using 1000 bootstrap replicates with 10 random additions and TBR branch

swapping. Previously published sequence information for in-group and out-group taxa is deposited under accession numbers AF092288-AF092421 (Hrbek & Larson 1999), and AY578711-AY578726 (Hrbek *et al.* 2004). Newly included taxa are *Kryptolebias sepia* (AY946272, AY946277), *Rivulus* aff. *urophthalmus* (AY946273, AY946278) and *R.* aff. *geayi* (AY946274, AY946279) from the type locality. Also newly included are *K. marmoratus* (AY946275, AY946280) and *K. brasiliensis* (AY946276, AY946281).

**Institutional abbreviation**—Institutional abbreviations are as given in Leviton *et al.* (1985) with the addition of NZCS (National Zoological Collection Surinam).

***Kryptolebias sepia*, n. sp.**

(Figs. 1, 2)

**Holotype**—ZMA 123.714 male, 61.8 mm: Surinam, Upper Marowijne system, Tapanahony River, 5 km downriver from Palumeu on the right bank, a hunting trail leads to a creek 15 minutes walk from the river; 03° 22' 43"N 055° 24' 41"W, coll. F.B.M. Vermeulen, 14 July 2003. Station number SU 2003-02.

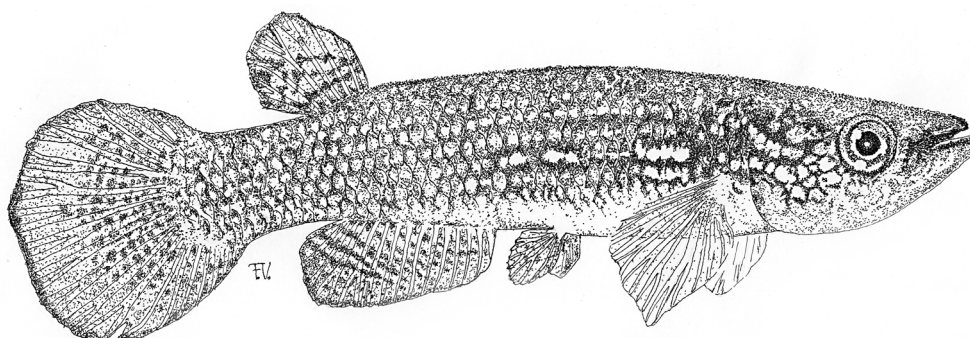
**Allotype**—ZMA 123.715 female, 65.1 mm. Surinam, Upper Marowijne system, Palumeu River 20 km upstream from the confluence of the Palumeu and Tapanahony Rivers, small creek 20 minute walk from riverbank, 03° 13' 51"N 055° 23' 46" W coll. F.B.M. Vermeulen, 16 July 2003. Station number SU 2003-06.

**Paratypes**—ZMA 123.716, 2 juveniles, 32.9–36.3 mm specimens collected by S. Sladkowsky and F.B.M. Vermeulen, 20 August 2000. Station number SVS 2000-02 (same collection locality as holotype). ZMA 123.717, 6 juvenile 31.5–38.1 mm specimens collected by F.B.M. Vermeulen, same collection data as holotype. NZCS F6418, (originally identified as *Rivulus cf. lanceolatus*, Eigenmann, 1909); Ulemari tributary, 13 km upstream of confluence with Litani River, 03° 13' 23"N 054° 15' 38"W, collected by P. E. Ouboter, S. Sahdew and S. Ramanand, 3–5 April 1998. NZCS F7063–F7066, 4 juvenile specimens, same collection data as holotype. MNHN 2004-3096, 1 specimen, same collection data as holotype.

**Diagnosis**—Distinguished from other *Kryptolebias* and *Rivulus* species by a combination of characters including low number of LL scales, high number of anal rays, short predorsal length and a robust body (Table 1), and unique mitochondrial DNA gene sequences. *Kryptolebias sepia* is further distinguished by remarkably strong melanistic pigmentation and the ability to change this coloration into several distinct patterns, lack of obvious sexual dimorphism, strong dichromatism between juveniles and adults, and absence of the “*Rivulus*” spot in females and juveniles.

**Description**—A medium sized *Kryptolebias*, robust shape, with a non-annual lifecycle. Morphometric data of holotype, allotype and eight paratypes from different localities are given in Table 2 in millimeters and in Table 3 as percent of standard length. Meristic data are in Table 4. Males differ little from females in their body coloration, shape and

fins. In adult males yellow marking on the gill cover is slightly more prominent and some but not all males show a few yellow markings along the lateral line. Frontal scalation pattern is in general E-type, but one individual showed mixed E-D-type (Hoedeman 1958). The strong melanism observed in *K. sepia* is also observed in at least three other species of *Kryptolebias*—*K. caudomarginatus*, *K. brasiliensis* and *K. marmoratus*—and in *R. atratus*. However, *K. sepia* is differentiated from these species by morphometric data, lower dorsal fin ray count (7.8 vs. 8.6 to 9.0), high anal fin ray count (15.1 vs. 11.2 to 12.6), by the absence of clear sexual dimorphism, by a network of dark markings beginning behind the eye and continuing just posterior of gill cover, by an oblique pattern on the lateral sides, and by their extraordinary capability to change color pattern within seconds. Diagnostic differences are given in Table 1. *Kryptolebias sepia* also differs by a strong orange body coloration of juveniles, which otherwise is only known in juvenile *R. amphoreus*. With the co-occurring *R. urophthalmus* / *R. stagnatus* group they share the lower number of dorsal rays and low number of LL scales but differ by a higher number of anal rays (15.1 vs. 12.3), by slightly shorter predorsal length to SL (76.6 % vs. 78.0 %), the absence of red spots forming lateral rows, and other diagnostic characters.



**FIGURE 1.** *Kryptolebias sepia* n. sp., adult male holotype ZMA 123.714 (drawing by F. B. M. Vermeulen).



**FIGURE 2.** *Kryptolebias sepia* n. sp., adult male from locality SU 2003-06 (type series).

**Adult males**—See Figures 1 and 2. Body grayish, dark brown near the dorsum, pale white near the belly. Scales on lateral sides show dark pigments forming a network pattern, scale centers are brighter forming five to six light rows from post operculum to the end of peduncle. Eight to 10 oblique bars are shown in subadults and adults depending on their mood. These oblique markings become more permanent and intense after reaching full maturity. Some scales along the lateral line on the anterior part of body show bright yellow color in sexually active males. Head dark brown to reddish brown, in the suprapectoral region a bluish shine is only visible with striking light, cheek pale white without markings. On the operculum, posterior to the eye, a network of deep black blotches start on partly yellow background forming two to three bands. On the lateral sides posterior to the gill opening the black blotches continue in an irregular branched pattern. All fins rounded without any filaments or extensions. Caudal fin shows a wine-red hue that is more intense near the caudal base. Around the caudal fin base a large number of small speckles, mostly situated along the rays, form a reticulated pattern. These reticulations become more intense with age. A dark wine-red margin is often present. Dorsal transparent with reddish hue and irregular wine-red dots, some speckles near the base form a reticulated pattern as in caudal but more subdued. Anal iridescent green, near the base fading to bluish white with numerous fine wine-red speckles forming five to six diagonal lines, more compact towards the fin tip. A wine-red band is weakly visible near the anal fin margin. Ventral fins iridescent pale blue, no markings. Pectorals clear but orange towards the base; color becomes more intense with age. Eyes with a golden-brown iris, more golden when sexually active.

**Adult females**—See Figures 3 and 4. Same coloration as in adult males. Body gray-brown toward the dorsum and whitish gray to white toward the ventrum. Depending on the mood of the female, eight to 10 dark melanistic oblique bars may appear or disappear. Also depending on mood, head black to grayish black, dorsal portion being reddish brown. Chin whitish, operculum black or with black markings on a not very distinct yellow background. All fins rounded without any filaments and with the same colors and patterns as adult males. Eyes same as in males. The so-called “*Rivulus*” spot is normally present on the dorsal portion of the caudal peduncle at its junction with the caudal fin, and is a major identifying feature of *Rivulus* females; however, it is not present in this species at any life-stage.

**Juveniles**—Head and body same as in adults, but the pale white color of the belly is replaced by orange white. Lateral pattern depends on the mood of the individual; see Figures 5, 6 and 7. During the night and when frightened, a fine oblique barred pattern appears following the lines of the scales. However, this pattern is also sometimes exhibited in other situations. Often pale golden blotches form an irregular pattern on the sides.

**Color in alcohol**—Males and females: body brown to yellow brown with barred pattern subdued but visible; chin, lower half of gill cover, and abdomen whitish; operculum with dark markings; unpaired fins with fine brown spots and markings.

**TABLE 1.** A comparison of key meristic characters of *K. sepioides* with other species of *Kryptolebias*, with sympatrically occurring *Rivulus* aff. *geayi* and *Rivulus* aff. *urophthalmus*, and with *R. obscurus* which also shows strong melanism. Data are based on Huber (1992) and Huber's www.killi-data.org.

	<i>K. sepioides</i> (N=10)	<i>K. marmoratus</i> (N=28)	<i>K. brasiliensis</i> (N=16)	<i>K. caudomarginatus</i> (N=26)	<i>R. geayi</i> (N=30)	<i>R. urophthalmus</i> (N=15)	<i>R. obscurus</i> (N=3)
Max size in cm	6.5	7.5	6.0	6.0	4.0	7.0	4.0
Dorsal rays	7.8 ± 0.4	8.6 ± 0.7	9.0 ± 1.1	8.9 ± 0.3	8.6 ± 0.6	7.2 ± 0.9	6.7 ± 0.5
Anal rays	15.1 ± 0.3	11.2 ± 0.9	12.6 ± 1.0	11.3 ± 0.3	11.7 ± 0.9	12.3 ± 0.9	9.8 ± 0.2
Dorsal to anal insertion	11.1 ± 0.3	8.2 ± 0.5	7.1 ± 0.9	8.0 ± 0.2	6.9 ± 0.5	10.0 ± 1.1	12.3 ± 0.6
Lateral line length in mm	39.0 ± 0.7	47.3 ± 2.0	31.5 ± 1.1	56.0 ± 3.1	32.5 ± 1.2	38.7 ± 1.6	30.3 ± 0.9
Predorsal length in % SL	76.6 ± 1.8	76.8 ± 1.7	73.5 ± 2.3	73.6 ± 0.8	71.3 ± 1.8	78.2 ± 1.3	78.7 ± 2.6
Frontal scalation type	D-E	E	E	E	F	E	E

TABLE 2. Morphometric data for *Kryptolebias sepioides* type series. All measurements are in mm.

	holotype		allotype		paratypes															
	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003	ZMA	SU 2003
<i>Kryptolebias sepioides</i>	123.714	123.715	123.716	123.717	123.718	123.719	123.720	123.721	123.722	123.723	123.724	123.725	123.726	123.727	123.728	123.729	123.730	123.731	123.732	123.733
Collection-item	SU 2003	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003	SVS 2000	SU 2003
Collection-item Loc/Sample nr.	02 (1)	06 (2)	02 (3)	02 (4)	02 (5)	02 (6)	02 (7)	02 (8)	02 (9)	02 (10)	02 (11)	02 (12)	02 (13)	02 (14)	02 (15)	02 (16)	02 (17)	02 (18)	02 (19)	02 (20)
Total length	61.8	65.1	32.9	36.3	37.8	31.7	35.2	38.1	31.5	32.1	40.2 ± 12.5									
Standard length	51.3	53.0	26.0	28.9	29.3	25.6	27.6	29.0	24.0	24.9	31.9 ± 10.8									
Greatest depth of body	10.6	9.8	5.1	5.4	6.4	5.3	5.7	5.4	4.9	4.8	6.3 ± 2.1									
Length of head	14.4	15.2	7.8	8.0	6.7	7.2	7.5	8.0	7.1	6.9	8.9 ± 3.1									
Eye diameter	3.6	3.8	2.3	2.3	2.1	2.2	2.4	2.4	2.1	2.2	2.5 ± 0.6									
Width of interorbital space	5.3	6.3	3.3	4.0	2.9	3.5	3.8	4.0	3.6	3.9	4.1 ± 1.0									
Length of snout	2.7	3.0	1.7	1.6	1.7	1.6	1.7	1.8	1.5	1.7	1.9 ± 0.5									
Length of caudal peduncle	7.7	7.9	4.0	5.0	5.3	4.4	5.5	5.3	4.3	4.3	5.4 ± 1.4									
Depth of caudal peduncle	7.2	6.9	3.3	3.6	4.1	3.3	3.8	3.5	3.0	3.0	4.2 ± 1.5									
Snout to dorsal insertion	39.4	41.5	20.0	22.2	22.8	18.7	21.4	21.3	18.6	19.2	24.5 ± 8.5									
Snout to anal insertion	30.9	33.3	16.2	18.7	18.5	15.5	17.8	18.1	15.1	15.2	19.9 ± 6.6									
Snout to ventral insertion	25.7	26.6	13.1	14.7	14.6	13.0	14.0	14.8	12.1	12.9	16.1 ± 5.4									



TABLE 3. Morphometric data for *Kryptolebias sepioides* type series. All measurements are in % of SL.

	holotype		allotype		paratypes													
	ZMA	123.714	ZMA	123.715	ZMA	123.716	ZMA	123.716	ZMA	123.716	ZMA	123.717	ZMA	123.717	ZMA	123.717	ZMA	123.717
<i>Kryptolebias sepioides</i>	SU 2003	SU 2003	SU 2003	SU 2003	SYS 2000	SYS 2000	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003	SU 2003
Collection-item	02 (1)	06 (2)	02 (3)	02 (4)	02 (5)	02 (6)	02 (7)	02 (8)	02 (9)	02 (10)	Average ± SD							
Collection-item Loc/Sample nr.	120.5	122.9	126.7	125.6	128.8	123.8	127.8	131.5	131.6	129.1	126.8 ± 3.7							
Total length	20.7	18.4	19.6	18.7	21.9	20.8	20.8	18.5	20.5	19.2	19.9 ± 1.2							
Greatest depth of body	28.1	28.6	30.2	27.5	23.0	28.1	27.1	27.7	29.6	27.7	27.8 ± 1.9							
Length of head	7.0	7.1	8.9	8.1	7.2	8.4	8.9	8.4	8.7	8.9	8.1 ± 0.8							
Eye diameter	25.0	24.7	29.3	29.3	31.2	29.9	32.7	30.2	29.4	32.1	29.4 ± 2.7							
Eye diameter in % to length of head	10.4	11.8	12.7	13.8	10.0	13.7	13.8	13.7	14.8	15.8	13.1 ± 1.9							
Width of interorbital space	5.3	5.6	6.5	5.4	5.7	6.3	6.0	6.3	6.3	7.0	6.0 ± 0.5							
Length of snout	14.9	14.9	15.4	17.3	18.2	17.3	19.8	18.3	18.0	17.3	17.1 ± 1.6							
Length of caudal peduncle	14.0	12.9	12.6	12.5	14.1	12.8	13.8	11.9	12.6	12.2	12.9 ± 0.8							
Depth of caudal peduncle	76.9	78.2	77.1	76.7	77.7	73.0	77.8	73.4	77.8	77.2	76.6 ± 1.8							
Snout to dorsal insertion	60.3	62.9	62.5	64.8	62.9	60.4	64.6	62.3	62.9	61.2	62.5 ± 1.6							
Snout to anal insertion	50.2	50.2	50.4	50.7	49.9	50.7	50.6	51.2	50.3	51.7	50.6 ± 0.5							
Snout to ventral insertion																		

TABLE 4. Meristic data for *Kryptolebias septia* type series.

	holotype		allotype paratypes																		
	ZMA	123.714	ZMA	123.715	ZMA	123.716	ZMA	123.716	ZMA	123.717	ZMA	123.717	ZMA	123.717	ZMA	123.717	ZMA	123.717	ZMA	123.717	
<i>Kryptolebias septia</i>	SU 2003	02 (1)	SU 2003	06 (2)	SVS 2000	02 (3)	SU 2003	02 (4)	SU 2003	02 (5)	SU 2003	02 (6)	SU 2003	02 (7)	SU 2003	02 (8)	SU 2003	02 (9)	SU 2003	02 (10)	
Collection-item	country/year																				
Collection-item	Loc/Sample nr.																				
<b>Meristic data</b>																					
Number of dorsal fin rays	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7	
Number of anal fin rays	16	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	
Dorsal insertion to anal insertion	+11	+11	+11	+11	+11	+11	+11	+11	+11	+11	+11	+11	+11	+12	+11	+11	+11	+11	+11	+11	
Number of ventral fin rays	6	6	6	6	7	7	7	7	6	6	7	6	6	6	6	6	6	6	6	7	
<b>Scalation</b>																					
Number scales lateral line + caudal fin	40+5	40+5	40+5	40+5	39+5	39+5	39+4	39+4	39+5	39+5	39+5	39+5	39+5	38+5	39+4	39+4	38+4	38+4	38+5	38+5	
Number scales to dorsal insertion	31	31	31	31	30	30	29	29	30	30	29	29	28	28	28	28	28	28	29	29	
Head scalation type	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	D-E	D-E	E	



**FIGURE 3.** *Kryptolebias sepia* n. sp., adult female with barred pattern from locality SU 2003-02 (type series).



**FIGURE 4.** *Kryptolebias sepia* n. sp., adult female with light pattern from locality SU 2003-02 (type series).

**Reproduction**—Eggs are 2 mm in diameter and have no major filaments on the cambium membrane, but are covered with an elastic and sticky substance. They are placed one by one between roots at the water's edge, or just above it. Under aquarium conditions, *K. sepia* deposits more than 50% of its eggs above the waterline. This behavior is occasionally observed in rivuline species of the genus *Rivulus* (FBMV pers. obs.). Very low water levels stimulate this species to mate, with males aggressively driving females. Decreasing water level to less than 3 cm instantly result in spawning activity. Color of eggs is dark amber matching the color of the spawning substrate. No differences in egg development time were observed under the three incubation conditions. In fully aquatic environment, eggs develop and hatch in 14 to 16 days at 24°C. On both wet and moist peat moss, eggs develop normally within the 14 to 16 day period, but do not hatch. If not allowed to hatch by immersing them in water, the embryos die within the following next seven days. This species is therefore unlikely to lay eggs capable of undergoing a developmental diapause (Wourms 1972), and the three week period potentially sets the upper time limit for temporary habitat desiccation. *Kryptolebias sepia* is also a good jumper and will, as many of its

family members do (Lüling 1971, Huber 1992), seek new water bodies by moving overland through leaf litter if its habitat becomes unsuitable.



**FIGURE 5.** *Kryptolebias sepia* n. sp., juvenile with light pattern.



**FIGURE 6.** *Kryptolebias sepia* n. sp., juvenile with barred pattern.



**FIGURE 7.** *Kryptolebias sepia* n. sp., juvenile with golden pattern.



**Habitat**—See Figure 8. *Kryptolebias sepia* appears to live only in extremely shallow parts of small creeks in hilly areas, and is restricted to primary forests with a very dense canopy cover. The actual habitat of *K. sepia* is found outside the main stream in swampy areas directly adjacent to the creeks themselves. The layer of leaf litter is often thick, sometimes over 50 cm, and is especially thick in the lower parts of creeks and adjacent flooded areas. *Kryptolebias sepia* is found in only a few centimeters of water which stands above this leaf layer, and over 95% of the population appears to consist of juveniles. In case of disturbance of the biotope, *K. sepia* seeks shelter in the soft bottom layer or jumps out of the water. The creeks proper had permanent running water. The water was clear, mineral poor, but with high concentrations of tannic acid, the so called black water type (Sioli 1984). No aquatic vegetation was present. Water temperatures were not always taken but likely never exceed 23°C; pH values varied between 4.1 and 5.4, and GH and KH hardness were both < 0.5 DH.



**FIGURE 8.** Biotope SU 2003-06 (after collecting activity) from which a portion of the type series comes.

*Kryptolebias sepia* is found syntopically with *Rivulus* aff. *urophthalmus* and frequently with *Rivulus* aff. *geayi*. On two occasions a third species of *Rivulus* was found together with *K. sepia*. This species was identified as *R. holmiae* by Hoedeman (1961) but moved to *R. igneus* by Huber (1991). This species, however, does not fit well with original

descriptions of either species, thus its true identity needs further study. The only non-rivulid fish fauna included the rare *Pyrrhulina laeta* and on one occasion *Tatia* aff. *creutzbergi*. Besides these species, no other fish were found in the creek or surrounding leaf litter.

It is likely *Kryptolebias sepia* is a predator of small fishes and invertebrates. However, aquarium observations suggest they are not cannibalistic. The authors' field observations suggest that in general all large and medium sized rivulids feed on tadpoles, ants and mosquito larvae and in many areas they probably contribute to controlling malaria outbreaks.

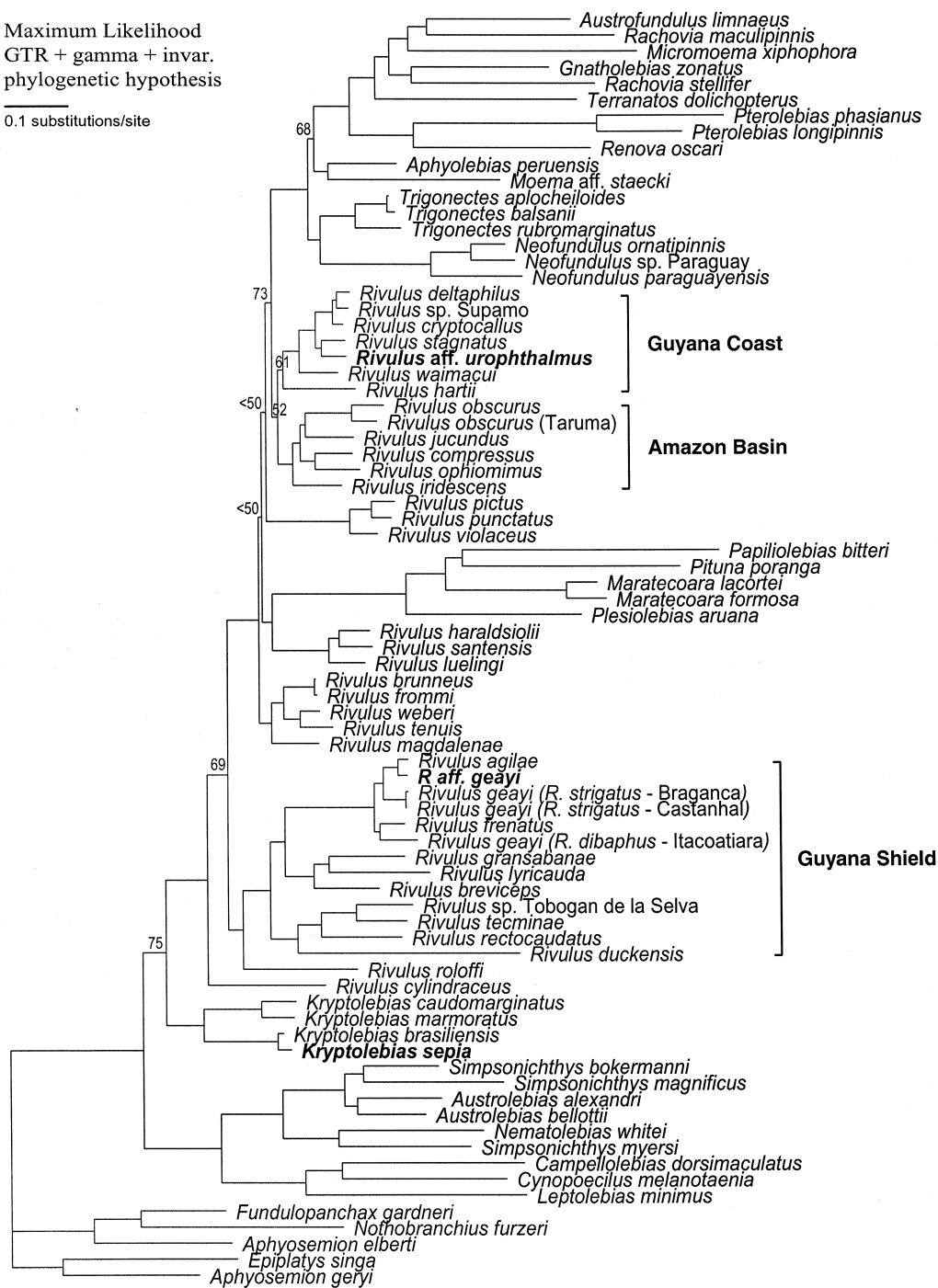
**Distribution**—*Kryptolebias sepia* was found only in creeks emptying into the Tapanahony and Palumeu Rivers and in the Ulemari River area (NZCS F 6418—fish collected by P. E. Ouboter, S. Sahdew and S. Ramanand in April 1998). Additional collections are needed, including the upper Litani and Marowini River basins, to confirm the authors hypothesis that *K. sepia* is wide spread in southeastern Surinam and southwestern French Guiana.

**Etymology**—The name *sepia* alludes to the family Sepiidae, the cuttlefish, whose chromatophores also can cause rapid changes in color.

**Phylogenetic relationships**—The maximum likelihood and the Bayesian likelihood analysis yielded identical topologies. Due to the size of the data set, it was not practical to estimate nodal support of the maximum likelihood topology via bootstrapping. Therefore, we report the maximum likelihood topology, conveying information on branch lengths, and indicate support for individual nodes with Bayesian posterior probability values (Fig. 9). *Kryptolebias sepia* shows a statistically well supported sister taxon relationship to *K. brasiliensis* (Figs. 9, 10). The monophyly of *Kryptolebias* whose members in addition to *K. sepia* are *K. marmoratus*, *K. caudomarginatus*, and *K. brasiliensis*, is also strongly supported (Figs. 9, 10). These relationships are recovered in all analyses, and strongly support the hypothesis that *K. sepia* is indeed a phylogenetic member of the genus *Kryptolebias*. Costa (2004b) also placed *R. campelloi* into *Kryptolebias*, although this species was not included in the analysis on which this taxonomic revision was based; it was reassigned without being listed as examined. The maximum parsimony topology (Fig. 10) suggests that *Kryptolebias* is sister to all other Rivulidae as hypothesized by Hrbek & Larson (1999), while the maximum likelihood topology (Fig. 9) suggests that *Kryptolebias* is sister to Rivulinae as hypothesized by Hrbek *et al.* (2004). The phylogenetic placement of *Kryptolebias* observed in the maximum likelihood hypothesis (Fig. 9) is also supported by Costa's (2004b) recent maximum parsimony analysis of morphological data. Thus the most likely phylogenetic hypothesis for *Kryptolebias* will need further investigation, but its monophyly and differentiation from *Rivulus* is undisputed. The two species (*Rivulus* aff. *geayi* and *Rivulus* aff. *urophthalmus*) with which *K. sepia* co-occurs, are members of a phylogenetically very distinct Amazonian clade of *Rivulus* (Hrbek & Larson 1999), and are not closely related to *K. sepia* (Figs. 9, 10).

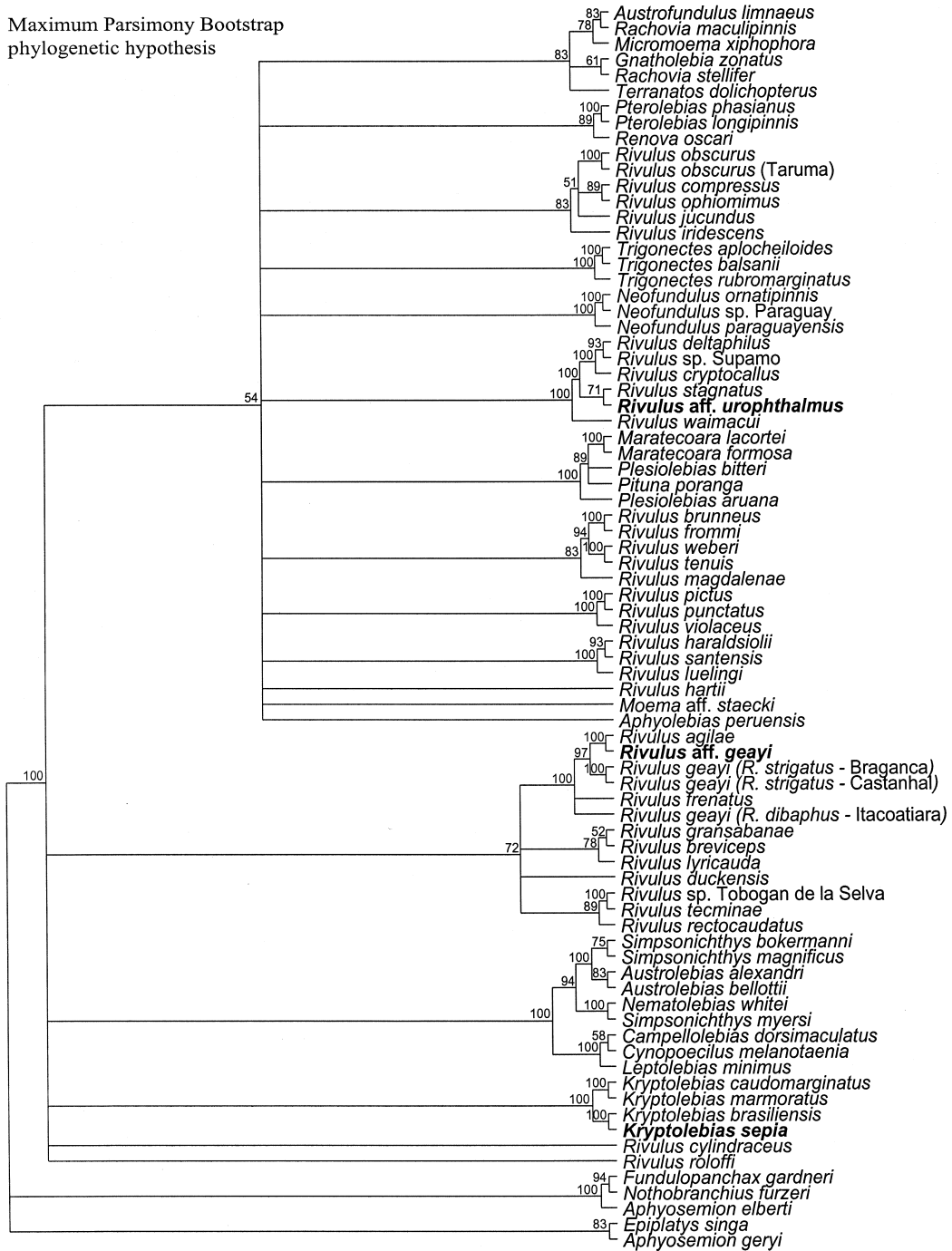
Maximum Likelihood  
GTR + gamma + invar.  
phylogenetic hypothesis

0.1 substitutions/site



**FIGURE 9.** Maximum likelihood estimate of phylogenetic relationships of the Rivulidae, and of *Kryptolebias sepia* to other rivulid species. In L = 60554.94372,  $\Gamma = 0.700598$ , pinvar = 0.137261. Generic names have been updated according to Costa (2003). Numbers above nodes represent Bayesian likelihood posterior probabilities; unlabeled nodes have values of at least 95%. *Kryptolebias sepia* as well as the two conspecific species (*R. aff. geayi* and *R. aff. urophthalmus*) are highlighted in **bold**.

Maximum Parsimony Bootstrap  
phylogenetic hypothesis



**FIGURE 10.** Maximum parsimony bootstrap phylogeny of the Rivulidae, and of *Kryptolebias sepia* to other rivulid species. Generic names have been updated according to Costa (2003). Numbers above nodes represent maximum parsimony bootstrap values. *Kryptolebias sepia* as well as the two conspecific species (*R. aff. geayi* and *R. aff. urophthalmus*) are highlighted in bold.



## Discussion

In the Tapanahony and Palumeu River drainages, as well as in other areas of northern South America there is a pattern of co-appearance of robust and small *Rivulus* species. The robust and the small species form reciprocally monophyletic groups which were labeled as the Guyana Coast and Guyana Shield clades, respectively (Hrbek & Larson 1999). Co-occurrence of robust and small species of *Rivulus* is usually observed in shaded primary forest of the mountainous highland plateau. These biotopes are characterized by very shallow, slowly running or stagnant water, low pH (3.5–5.1), low mineral concentrations (hardness < 0.5 DH), and thick canopy with diffuse light. These marginal habitats are unoccupied by other fish species except for occasional *Pyrrhulina* spp.

Typical community compositions include *R. igneus* with *R. cladophorus* or *R. geayi*; *Rivulus* aff. *igneus* with *Rivulus* aff. *geayi*; *R. waimacui* with *R. breviceps* or *R. sp.* Mahdia; and *R. immaculatus* with *R. gransabanae* or *R. torrenticola*. Sister clade to the Guyana Coast is the Amazon basin clade (Hrbek & Larson 1999) whose members form similar associations with the eastern Guyana Shield subclade (Hrbek *et al.* 2004). These associations include *R. ophiomimus* with *R. rectocaudatus* and *R. compressus* with *R. duckensis*. However, even within these communities, the robust species tends to be found in flooded swampy parts of the creek, while the smaller species tends to be found in the creek proper. Naturally some species within these clades do not fit this ecological pattern. These species include, for example, *R. agilae* which prefers open savannah and secondary forest habitats, and usually occurs alone, or *R. hartii* which is found in a wide variety of habitats but seem to usually co-occur with poeciliids for which they are a major predator (Reznick 1982). The *R. urophthalmus* species complex, which includes *R. urophthalmus*, *R. stagnatus*, *R. deltaphilus* and *R. lungi* among others, occurs with rivulids as well as poeciliids.

The newly described *Kryptolebias sepia* also fits within this pattern of co-occurrence. Although it superficially resembles robust *Rivulus* species, it is a member of a phylogenetically distinct clade, and appears to be ecologically differentiated as well. Formal taxonomic recognition of this clade did not occur until recently when Costa (2004b) described the genus *Kryptolebias*. Costa (2004b) reanalyzed *Rivulus brasiliensis* and its close relatives—all species analyzed in Costa (1998) as well—and no longer found *R. brasiliensis* and its relatives to have synapomorphies which previously placed it within a monophyletic *Rivulus* (Costa 1998). This made *Rivulus* non-monophyletic, a hypothesis that has been put forth by molecular phylogenetic analyses (Hrbek & Larson 1999, Murphy *et al.* 1999), and by an unpublished molecular study reviewed by and cited in Costa (1998). Costa (2004b) therefore proposed the new generic name *Cryptolebias* which, however, is preoccupied by a fossil cyprinodont fish (Gaudant 1978). As first reviser, Costa (2004a) substituted the name *Kryptolebias* for *Cryptolebias*. In spite of a somewhat complicated morphological history, all lines of evidence now clearly show that *Kryptolebias* is a clade distinct from the remaining *Rivulus* species. Whether the remaining *Rivulus* species form a clade (Costa 1998, 2004b) or are non-monophyletic (Hrbek & Larson 1999, Murphy *et al.*

1999, Hrbek *et al.* 2004) remains to be resolved, but the taxonomic validity of *Kryptolebias* is upheld by this study.

## Conclusion

*Kryptolebias sepia* is a distinct taxon that is morphologically, ecologically and phylogenetically differentiated from sympatrically occurring robust (*Rivulus* aff. *igneus* or *R. urophthalmus*) and small (*Rivulus* aff. *geayi*) species. It occurs in low densities in a rarely collected primary forest habitat of the central mountains of Surinam, and is easily overlooked in usual surveys. This study clearly shows that a better understanding of rivuline biodiversity, and of the genera *Rivulus* and *Kryptolebias* specifically, will require a focused collecting effort in appropriate habitats, as well as the combined use of molecular and morphological data in taxonomic and phylogenetic studies.

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