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Meristic and Morphometric Differences in Populations of *Rivulus marmoratus*

D. Scott Taylor

Rivulus marmoratus **(Pisces: Aplocheilidae) is a small cyprinodontid found in mangroves of the western tropical Atlantic and is the only known self-fertilizing, hermaphroditic vertebrate. Populations normally consist of genetically diverse groups of homozygous clones.** ***Rivulus marmoratus*** **has the widest range of any member of the genus (southern Brazil to central Florida) and is the only marine representative of the genus. There has been considerable speculation about the "origin" of the species in an otherwise sexually reproducing genus and family. Although well studied in the laboratory, few specimens have been collected from the wild until recently. This study examined meristic and morphometric differences among 12 widespread populations and among individual clonal lineages reared in the laboratory. Thirty-two meristic and morphometric characters in 187 fish (ranging from Brazil to Florida) were examined with univariate and multivariate statistics for determination of overall differences among populations. To control for possible environmental effects, offspring of eight clones from two populations (Belize and Florida) were reared at 25 C and similarly analyzed. All characters with the exception of pectoral fin rays were significantly different among wild populations. In addition, Belizean male fish differed from hermaphrodites in several morphometric characters. Fish reared at constant temperature also displayed significant differences, indicating a probable genetic component to differences in wild fish. The multivariate analyses also confirmed considerable heterogeneity among** ***R. marmoratus.*** **Overall, fish from Brazil, the Florida Everglades, Belize, and Honduras appear to be distinct from the other groups. The characters that contributed most to group delineations were number of precaudal vertebrae, distance from pelvic fin origin to anal fin origin, pectoral fin length, and number of branched caudal rays. Discriminant function analysis was able to classify the fish reared at 25 C to individual clonal heritage, although with a different set of characters than in wild fish.**

Analysis of meristics and morphometrics (counts of serially repeated body structures and relative body measurements) has been used in a variety of applications for the study of fishes. Variation of meristic and morphometric traits within a species or between closely related species has been attributed to the combination of environmental and genetic factors interacting on developing embryos (Fowler, 1970). This interaction can lead to a great deal of phenotypic variation within related groups or species. Phenotypic plasticity, or the potential for a character to change in response to environmental influences, is a widespread phenomenon in fishes, and much morphological variation even within a species in the same habitat can result (Ehlinger and Wilson, 1988).

Among fishes, a model organism that allows separation of environmental vs genotypic influences on meristic and morphometric characters in the laboratory would be desirable. The discovery of *Rivulus marmoratus* (Poey), the only known self-fertilizing vertebrate animal (Harrington, 1961), has provided such a model. In the wild, *R. marmoratus* populations most frequently exist as diverse arrays of homozygous clones, although two heterozygous populations have been identified, and uniparental descendants of wild-caught hermaphrodites are uniformly homozygous, isogenic clones (Kallman and Harrington, 1964; Harrington and Kallman, 1968; Turner et al., 1992b; Lubinski et al., 1995; Taylor, 2001; Taylor et al., 2001). This genetic uniformity has led to laboratory studies on the influence of temperature on vertebral counts, meristic variation (Lindsey and Harrington, 1972; Harrington and Crossman, 1976a, 1976b; Swain and Lindsey, 1986b), and meristic variation as influenced by parental reproductive history (Swain and Lindsey, 1986a).

An additional interesting observation of temperature studies on *R. marmoratus* was the discovery that low temperature during egg development or exposure of young hermaphro-

Fig. 1. Map of collection sites for 11 populations of *Rivulus marmoratus*.

dites to high temperature can produce male fish (Harrington, 1967). Since this laboratory observation, male fish have been found only rarely in the wild, with the exception of a high proportion (10–25%) of the population on some Belize, Central America cays (Davis et al., 1990; Turner et al., 1992a). On the Belize Cays, males apparently participate in sexual reproduction by means of outcrossing, as evidenced by the uniform heterozygosity of fish from there (Lubinski et al., 1995). A lower level of heterozygosity (~28%) has also been documented from the Bay Islands, Honduras (Taylor et al., 2001).

In spite of the extensive laboratory work cited above, meristic and morphometric studies on wild-caught *R. marmoratus* are lacking. Harrington's work was centered on three clones

TABLE 1. *Rivulus marmoratus* collection sites, number of specimens examined, date of collection, site code, habitat type and source of specimens.

Location	N	Date of collection	Site code	Habitat code[a]	Source
Vero Beach, Florida	4	1988–89	V	1	D. S. Taylor
St. Lucie County, Florida	20	1995	S	1, 2	D. S. Taylor
West Palm Beach, Florida	3	1986	W	2	R. Whitman
Long Key, Florida	10	1995	L	1	D. S. Taylor
Flamingo, Florida (Everglades)	20	1995	E	2	D. S. Taylor
Naples, FL	20	1992, 1997	N	2	S. Ritchie, D. Addison
Twin Cays, Belize (hermaphrodite)	30	1992	BL	1	D. S. Taylor
Belize Cays, Belize (male)	11	1992	BLM	1	D. S. Taylor
Utila, Bay Islands, Honduras	10	1996	H	1	D. S. Taylor
Norman's Pond Cay, Exumas, Bahamas	30	1997	B	1	D. S. Taylor
Rio de Janeiro, Brazil	23	1989	BZ	3	American Museum of Natural History
Mangaratiba, Brazil (Sao Paulo)	6	1975–86	SP	4	University of Sao Paulo

[a] Habitat code: 1, crab burrows; 2, intermittently flooded swale/slough; 3, shallow grassy pond in mangroves; 4, unknown.

TABLE 2. Capture sites, specimen codes, and number of *Rivulus marmoratus* offspring reared at 25 C.

Parental capture site	Specimen code (generation)	No. of offspring reared
Floridana Beach, Florida	CH (F3)	10
Melbourne Beach, Florida	SSH (F3)	9
New Symrna Beach (Volusia County), Florida	VOL (F2)	6
Belize (Papa Gabriel) Twin Cays, C.A.	PG3 (F1)	4
Belize (Papa Gabriel) Twin Cays, C.A.	PG6 (F1)	3
Belize (Papa Gabriel) Twin Cays, C.A.	PG8 (F1)	6
Belize (Papa Gabriel) Twin Cays, C.A.	PG10 (F1)	6
Belize (Papa Gabriel) Twin Cays, C.A.	PG12 (F1)	6
	Total	50

from Vero Beach, Florida, and his field collections numbered fewer than 50 fish, of which he tabulated meristics on 20 specimens (Harrington and Rivas, 1958; R. W. Harrington, unpubl. data). This remains the only examination of meristics on any number of wild-caught *R. marmoratus,* other than that of the type specimens (Rivas, 1945), several specimens from various museums (Huber, 1992), and 10–15 specimens from Venezuela (Taphorn, 1980). A single wild fish from Miami, FL, was examined by Lindsey and Harrington (1972) and was found to have lower vertebral and pectoral fin ray counts than Vero Beach fish. They concluded that because this population was subject to higher ambient temperatures than the Vero Beach population, fish from Miami may "... differ with regard to meristic variation."

Rivulus marmoratus is found from southern Brazil to central Florida and has the widest range of all members of the genus (Davis et al., 1990; Taylor, 1993). In a phylogenetic analysis [mitochondrial DNA (mtDNA)] of 16 northern species of the genus *Rivulus,* Murphy and Collier (1996) suggested that the origin of the genus is South American. Two freshwater *Rivulus* species from the greater Antilles were of vicariant origin, isolated by the breakup of the temporary land bridge between North and South America about 70–80 million years ago. Two sister groups (Central American–Columbian clade and northern Venezuelan–Guiana clade) were also identified. Lesser Antilles species appear to have arrived by recent dispersal from coastal South America. Murphy (1997) found that *R. marmoratus* is most closely related to *R. caudomarginatus,* a salt-tolerant, gonochoristic species sympatric with *R. marmoratus* in Brazil (Huber, 1992). Huber (1992) reached the same conclusion, based on very limited meristic data.

Because of its wide latitudinal distribution and existence in both heterozygous and homozygous forms, *R. marmoratus* may offer some insights into questions on the effects of clonal reproduction on meristic and morphometric variation, biogeography, and the relationships among widespread populations. In addition, the selfing mode of reproduction allows separation of genotype vs environmental influences in captive-reared specimens, which provides the unique opportunity to examine physical variation among individual clonal lineages. This study explores these issues by examining both wild populations and laboratory-reared known clonal lineages.

MATERIALS AND METHODS

Specimen collection and measurement.—Meristics and morphometrics were examined in 187 wild *R. marmoratus* hermaphrodites from 11 separate populations (Fig. 1; Table 1). Standard length data were collected from an additional 344 specimens from eight of the 11 populations. In addition, morphometric data were collected from a population of 11 phenotypic male fish from the Belize Cays for comparison with Belize hermaphrodites. Specimens were collected using hook and line, traps inserted into crab burrows, dip net, or wire minnow traps (Taylor, 1988, 1990) or were provided on loan from collections at the American Museum of Natural History and the University of Sao Paulo, Brazil. Fish collected by the author were anesthetized in 2-phenoxy-ethanol, fixed for 2 d in buffered 10% formalin, rinsed in freshwater, and placed in 70% ethanol for storage.

An experiment was also designed to control for temperature differences between two geographic areas and for examination of phenotypic differences between known clonal lineages. Parental fish from east-coast Florida and Twin Cays, Belize, were kept at 25 ± 4 C, 12:

TABLE 3. Mean and SD for 12 meristic counts in 11 populations of *Rivulus marmoratus*. Meristic codes are provided in the text and population site codes in Table 1.

Fish site	DR	ANR	CRBR	CRR	PCR	PLR	LSC	TRSC	PDSC	CPSC	PCVR	CVR
BL												
Mean	8.53	11.77	13.10	15.5	13.17	6.30	47.33	15.10	35.23	11.22	12.33	19.1
SD	0.57	0.57	0.92	1.9	0.38	0.41	1.83	0.44	2.14	0.41	0.48	0.6
V												
Mean	8.75	11.25	15.25	13.7	13.25	6.38	48.75	15.63	34.75	11.75	12.50	19.7
SD	0.43	0.43	0.43	0.8	0.25	0.41	0.56	0.41	1.48	0.25	0.50	0.4
S												
Mean	8.85	11.70	14.80	14.6	13.10	6.28	47.10	14.98	35.85	11.13	12.60	19.5
SD	0.49	0.57	1.06	1.1	0.26	0.57	1.55	0.26	1.98	0.28	0.60	0.6
W												
Mean	8.67	12.00	15.00	15.0	13.00	6.50	49.33	15.00	35.00	11.00	13.00	19.6
SD	0.58	0.00	1.00	1.0	0.00	0.50	1.53	0.00	2.00	0.00	0.00	0.5
E												
Mean	9.15	12.60	14.00	14.8	13.00	6.75	48.03	15.55	37.10	11.10	13.95	19.1
SD	0.59	0.68	0.00	0.7	0.00	0.44	1.62	0.46	1.77	0.26	0.22	0.3
L												
Mean	9.10	11.40	13.94	16.3	13.15	5.70	46.90	15.15	35.10	11.10	12.85	19.1
SD	0.57	0.52	1.39	1.0	0.33	0.71	1.39	0.47	1.52	0.32	0.37	0.4
N												
Mean	8.80	11.60	14.65	15.2	13.15	6.48	50.78	16.30	37.60	11.80	12.90	19.1
SD	0.41	0.50	0.93	1.0	0.37	0.44	2.59	0.57	2.64	0.66	0.31	1.1
B												
Mean	9.07	11.67	13.97	16.9	13.02	6.13	48.23	15.80	34.87	11.42	12.80	18.4
SD	0.37	0.48	0.72	1.1	0.09	0.77	1.87	0.45	1.41	0.42	0.41	0.5
H												
Mean	8.60	11.30	13.20	15.6	13.05	6.75	43.50	15.10	31.20	10.85	13.00	18.6
SD	0.52	0.48	0.92	0.7	0.37	0.42	1.33	0.46	1.40	0.34	0.00	0.5
BZ												
Mean	8.43	11.74	16.22	14.3	12.89	6.83	47.04	15.67	34.70	11.41	12.74	19.1
SD	0.51	0.69	0.52	0.8	0.34	0.47	1.96	0.44	1.22	0.47	0.45	0.5

TABLE 3. Continued.

Fish site	DR	ANR	CRBR	CRR	PCR	PLR	LSC	TRSC	PDSC	CPSC	PCVR	CVR
SP												
Mean	8.43	11.86	16.14	15.2	13.00	6.71	49.08	15.58	35.33	11.25	13.00	19.3
SD	0.53	0.38	1.21	1.5	0.00	0.39	1.20	0.80	1.75	0.42	0.00	0.5
ANOVA												
P value	≤0.05	≤0.05	≤0.05	≤0.0	ns[a]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.0

[a] NS, not significant.

12 light–dark photoperiod in 11.4-cm-diameter glass finger bowls with 300 ml of 25 ppt seawater. A plastic screen across the bottom of the bowl prevented egg cannibalism (Koenig and Chasar, 1984). Fish were fed 5 d/wk with 1- to -3-d-old *Artemia* or mosquito larvae. Bowls were checked daily for eggs that were removed and placed in an incubator at 25 ± 2 C until hatching. Hatchlings were immediately transferred to an environmental chamber (25 ± 3 C) in finger bowls with 300 ml of 25 ppt seawater. Twenty-five offspring (F1–F3) of each set of wild parental fish were reared. The offspring were fed ad libitum 7 d/wk on 1- to 3-d-old *Artemia,* and water was changed every 3 wk. At age 90 d, the approximate age of sexual maturity (Kristensen, 1970; Koenig and Chasar, 1984; Davis, 1986), fish were anesthetized and fixed as above. Table 2 shows the "lines," designated from single, wild-caught parental fish that were reared at 25 C.

The following 19 morphometric characters for all wild-caught and laboratory-reared fish were measured to the nearest 0.1 mm with needle point vernier calipers under a dissecting microscope. Measurements follow those of Hubbs and Lagler (1958) and Rivas (1944) except where noted: standard length (SL), total length (TL), predorsal length (PDLN), preanal length (PALN), length of dorsal fin base (DBSLN), dorsal fin length (DLN), caudal length (CLN), length of anal fin base (ABSLN), anal length (ALN), pectoral fin length (mean of left plus right) (PCLN), pelvic fin length (mean of left plus right) (PLLN), distance from pelvic fin origin to anal fin origin (POAO), pelvic fin base (mean of left plus right) (PLBS), body depth (BDP), depth of caudal peduncle (CPDP), head length (HDLN), head width (HDWD), interorbit distance (INORB), and orbit diameter (left eye only) (ORBD).

Meristic counts, following the methods of Hubbs and Lagler (1958) and Rivas (1944), were also made on all specimens viewed under a dissecting microscope. The wild specimens were also x-rayed for vertebral counts and confirmation of rudimentary ray counts. No meristic counts or x-rays were taken from the 11 wild Belize male fish. The full suite of morphometric measurements were made on the 50 specimens reared at 25 C. They were then stained with alizarin red to facilitate rudimentary ray and scale counts. No x-rays were taken of these specimens. Fin ray counts include all elements, with the exception of branched and rudimentary caudal rays that were counted separately, and rays split to the base were counted as two. Counts

TABLE 4. Mean and SD for 19 morphometric measurements in 12 populations of *Rivulus marmoratus*, including hermaphrodites and males from Belize. Morphometric codes are provided in the text and population site codes in Table 1. Measurements are expressed in percent standard length.

Fish site	SL (mm)	TL (mm)	PDLN	PALN	DBSLN	DLN	CLN	ABSLN	ALN
BL									
Mean	27.96	33.92	0.76	0.63	0.09	0.18	0.21	0.14	0.24
SD	4.35	5.24	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BLM									
Mean	29.38	36.32	0.76	0.63	0.09	0.20	0.24	0.15	0.26
SD	4.73	5.83	0.02	0.02	0.01	0.01	0.01	0.01	0.01
V									
Mean	35.68	43.65	0.75	0.65	0.10	0.20	0.22	0.15	0.24
SD	3.81	4.84	0.01	0.02	0.00	0.01	0.01	0.01	0.01
S									
Mean	31.42	38.18	0.76	0.63	0.09	0.19	0.22	0.14	0.24
SD	4.33	5.13	0.01	0.01	0.01	0.01	0.02	0.01	0.01
W									
Mean	30.10	37.13	0.75	0.63	0.09	0.20	0.23	0.14	0.25
SD	1.13	1.03	0.01	0.01	0.01	0.00	0.01	0.00	0.01
E									
Mean	28.71	35.17	0.75	0.63	0.10	0.23	0.30	0.15	0.23
SD	2.21	2.63	0.01	0.01	0.01	0.04	0.07	0.01	0.01
L									
Mean	25.81	31.94	0.76	0.63	0.10	0.20	0.24	0.14	0.25
SD	3.13	3.70	0.01	0.01	0.01	0.01	0.01	0.00	0.01
N									
Mean	32.94	40.21	0.75	0.63	0.10	0.20	0.22	0.14	0.25
SD	5.13	5.91	0.01	0.01	0.01	0.01	0.02	0.01	0.01
B									
Mean	28.14	34.95	0.76	0.63	0.09	0.20	0.24	0.14	0.25
SD	4.91	6.14	0.01	0.01	0.01	0.01	0.01	0.01	0.01
H									
Mean	24.16	29.79	0.75	0.64	0.09	0.20	0.24	0.14	0.26
SD	3.51	4.04	0.01	0.01	0.00	0.01	0.02	0.01	0.01
BZ									
Mean	27.83	34.59	0.76	0.62	0.09	0.19	0.24	0.14	0.24
SD	4.08	5.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SP									
Mean	31.82	39.38	0.75	0.63	0.10	0.20	0.24	0.14	0.24
SD	4.73	5.93	0.01	0.01	0.01	0.01	0.01	0.01	0.01
ANOVA *P* value	≤0.05		≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05

are dorsal rays (DR), anal rays (ANR), branched caudal rays (CRBR), rudimentary caudal rays (CRR), pectoral rays (mean of left plus right) (PCR), pelvic rays (mean of left plus right) (PLR), scales in lateral series (mean of left plus right—from upper pectoral fin axil to end of hypural plate) (LSC), transverse scale rows (from dorsal origin in a diagonal row to anal origin—mean of left plus right) (TRSC), predorsal scales [counted from the most posterior frontal scale "B" (Hoedeman, 1958) to the dorsal fin origin] (PDSC), scales around the caudal peduncle (mean of left plus right) (CPSC), precaudal

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TABLE 4. Extended.

PCLN	PLLN	POAO	PLBS	BDP	CPDP	HDWD	HDLN	INORB	ORBD
0.18	0.07	0.09	0.02	0.17	0.13	0.20	0.28	0.13	0.08
0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00
0.20	0.07	0.08	0.02	0.18	0.13	0.21	0.29	0.13	0.08
0.01	0.01	0.01	0.00	0.02	0.01	0.01	0.01	0.01	0.00
0.20	0.08	0.08	0.02	0.18	0.14	0.20	0.27	0.13	0.07
0.01	0.02	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00
0.18	0.07	0.08	0.02	0.19	0.14	0.21	0.27	0.12	0.07
0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00
0.21	0.08	0.08	0.02	0.16	0.12	0.27	0.28	0.13	0.08
0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
0.19	0.07	0.08	0.02	0.17	0.13	0.23	0.27	0.12	0.07
0.00	0.01	0.00	0.00	0.01	0.00	0.03	0.01	0.01	0.01
0.20	0.07	0.08	0.02	0.16	0.13	0.21	0.29	0.13	0.08
0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01	0.00
0.18	0.07	0.07	0.02	0.18	0.13	0.21	0.27	0.12	0.07
0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00
0.20	0.07	0.08	0.02	0.18	0.13	0.22	0.28	0.13	0.08
0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00
0.20	0.08	0.09	0.02	0.17	0.13	0.20	0.29	0.14	0.08
0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.01
0.19	0.07	0.08	0.01	0.16	0.12	0.20	0.27	0.12	0.08
0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01
0.18	0.08	0.08	0.02	0.17	0.12	0.20	0.28	0.12	0.08
0.01	0.01	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00
≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05

vertebrae (PCVR), caudal vertebrae (CVR), and total vertebrae (TOTV). Some specimens were missing one or both pelvic fins, and if only one fin was missing, the ray count (or length) of the single fin was used. Specimens with both fins missing were eliminated from the analysis.

Data analysis.—Individual morphometric measurements were converted to percent standard length for illustration and for analysis by conventional parametric or nonparametric statistics (i.e., between Belize males and hermaphrodites). Analysis of variance (ANOVA), two-tailed t-tests, and Mann–Whitney rank sum tests with sequential Bonferroni corrections were used to determine whether individual characteristics differed among populations.

To determine whether the total meristic and morphometric data set indicated phenotypic differences among sites, the data were pooled for each population, and the 11 populations were analyzed using principal components analysis (PCA). In PCA, meristic counts were entered untransformed into a correlation matrix. Morphometric variables (raw measurements) were log transformed and run in a covariance matrix. The 50 fish raised at 25 C were analyzed using discriminate function analysis (DFA), with the intent of determining whether specimens could be assigned to a specific clone. Because of sample size limitation (number of variables must be less than or equal to n/3) (Williams and Titus, 1988), the raw meristic and morphometric variables were analyzed separately.

Results

Meristic and morphometric data for wild hermaphrodites.—Examination of the meristic and morphometric data (Tables 3, 4) indicates that individual characters exhibit significant variability among the 11 hermaphroditic populations studied. ANOVA indicates that the differences among sites are significant ($P \leq 0.05$) for all meristic counts except pectoral rays ($F_{10,176} = 2.00$, $P = 0.058$) and for all morphometric measurements ($P \leq 0.05$).

For eight of the 11 hermaphroditic *R. marmoratus* populations, the standard length of 344 additional specimens was measured to achieve a larger sample size for determination of overall size differences among populations. Data for the four sites without additional specimens (West Palm Beach, Belize males, Brazil, and Sao Paulo) were retained for inclusion in the broader analysis. When mean standard length of the 10 hermaphroditic populations (with Brazil and Sao Paulo combined) is regressed against latitude of collection site, there is a marginally significant increase in standard length with increasing latitude (df = 8; $P = 0.05$; $R^2 = 0.44$), although collection techniques were not uniform among all collections.

Morphometric data for hermaphrodite and male fish from Belize.—The collection of a number of phenotypic male *R. marmoratus* in Belize provided a rare opportunity to compare the structure of the two phenotypes (Table 4). Males had significantly longer anal, dorsal, pectoral, and caudal fins than hermaphrodites, and the base of the anal fin was also longer. Hermaphrodites had a longer distance from pelvic fin origin to anal fin origin.

Meristic and morphometric differences among fish reared at 25 C.—Rearing two distinct genetic lines of fish, from Florida and Belize, at a constant temperature of 25 C allowed testing of the hypothesis that the differences in meristic and morphometric characters observed among these two populations have a genetic basis. The meristic data indicate that there are genetic differences in several traits (Table 5). Sequential Bonferroni t-tests indicate that mean rudimentary caudal ray counts were higher in Belize fish, whereas there were more branched caudal rays in Florida fish. Florida fish also had greater lateral, transverse, and caudal peduncle scale counts. Significant differences were also found in three morphometric measurements (Table 6). Florida fish had a greater length of dorsal and pelvic fin bases, and Belize fish had a greater caudal fin length.

In addition to quantifiable differences among the meristic and morphometric parameters, certain physical anomalies were noted among some of the 25 C specimens. These included kyphosis (downward or upward curvature of spine), a "notch" in the dorsal tissue just anterior to the caudal peduncle or anterior to the dorsal fin origin, and a permanent outward "flare" of the ventral portions of the operculum. Four Belize (PG) specimens displayed two symptoms (gill flare and notch at caudal peduncle), whereas nine Belize specimens had only one symptom. Overall, four of 25 Florida offspring and nine of 25 Belize fish were deformed.

Wild fish–combined populations: PCA.—The analysis of meristic data identified three factor loadings that explained 54% of the variation in the data (Table 7). In PCA, each individual is assigned a "score" for each factor, which is the sum of the component loadings for each variable after standardization in the correlation matrix. Scatter plots of each specimen's factor scores provide a graphical analysis of the similarities and differences of individual fish. Although there is a great deal of overlap in meristic factor scores 1 and 2 (Fig. 2), fish from Naples (N) were generally grouped in one sector of the plot, with high positive values for factor 1, which separates them as having greater scale counts. Both Brazil (BZ) and Sao Paulo (SP) have high negative factor 2 scores, interpreted as having more branched caudal rays and pelvic rays and fewer rudimentary

TABLE 5. Mean and SD for 10 meristic counts of 50 offspring from Florida (FL) and Belize (PG) *Rivulus marmoratus* raised at 25 C, with Bonferroni-corrected Mann–Whitney rank sum test results. Meristic codes are provided in the text.

	DR	ANR	CRBR	CRR	PCR	PLR	LSC	TRSC	PDSC	CPSC
FL										
Mean	8.72	11.52	13.52	14.84	13.74	6.82	49.02	15.86	34.96	11.36
SD	0.45	0.51	0.82	1.28	0.35	0.36	1.73	0.27	1.30	0.37
PG										
Mean	8.60	11.36	11.88	16.60	13.72	6.72	47.04	15.30	34.68	11.08
SD	0.50	0.49	1.09	1.50	0.61	0.33	1.83	0.38	1.44	0.24
Mann–Whitney T value	675.00	687.50	865.00	439.50	651.00	700.50	808.50	856.50	662.50	774.00
Bonferroni *P* value	1.41	1.36	0.009*	.01*	0.80	1.11	0.008*	0.007*	1.26	0.048*

* Significant difference at $P \leq 0.05$.

caudal rays (Fig. 2). The plot of factor scores 1 and 3 showed differentiation for Everglades (E), which groups clearly with higher anal ray and dorsal ray and total vertebrae counts (Fig. 3). The meristic score of factor 1 for each fish can be regressed against the latitude of its collection site (Fig. 4). In this example, there is a significant relationship between PCA meristic scores and latitude (df = 172; $R^2 = 0.21$; $P < 0.001$). The strongest relationship between increasing meristic counts and increasing latitude was for the four scale counts (Table 7). Caudal rays (branched and rudimentary) contributed most significantly to factor 2, whereas total vertebrae, dorsal, and anal fin rays dominated loadings for factor 3.

A similar analysis of morphometric data indicates that the first three factors explain 95% of the variance in the data, with factor 1 accounting for nearly 87% (Table 8). Because the loadings in factor 1 for the morphometric analysis are uniformly high and nearly equal, this factor can be attributed to general differences in fish size in the sample (Shepherd, 1991). The scatter plot of scores of the overall morphometric analysis for factors 1 and 2 (not shown) fails to clearly separate any group. Although factors 2 and 3 account for less than 8% of the total variance, this plot (Fig. 5) begins to separate fish from Belize, Brazil, and Sao Paulo. Belize fish have a greater distance between pelvic fin origin and anal fin origin and shorter length of dorsal fin base. Both Brazil and Sao Paulo fish have a tendency toward larger fins: greater pectoral fin length, dorsal length, and caudal length; a wider length of dorsal base; greater orbit diameter; and a much smaller pelvic fin base.

Florida–Belize fish reared at 25 C: DFA.—In these analyses, fish were identified by individual clones (n = 8) to examine the capability of DFA to discriminate to this level. A significant difference was found among the eight groups in meristics (Wilks' lambda = 0.01; F = 3.42; df = 10,33; $P < 0.001$). Groups were classified at 96% and 48% (jacknifed). Figure 6 depicts the plot of scores 1 and 2, and the most similar clone pairs were 1) VOL–SSH; 2) PG8–PG6; and 3) SSH–PG3, whereas the most dissimilar clone pairs were: 1) CH–PG10; 2) SSH–PG12; and 3) PG3–PG12. There is a clear indication of individuals within a single clone clustering together. The two characters most useful in the discrimination were pectoral fin rays and scales in lateral series.

For the morphometric variables, classification was 96% and 46%, differences were again

TABLE 6. Mean and SD for 19 morphometric measurements of 50 offspring of Florida (FL) and Belize (PG) *Rivulus marmoratus* raised at 25 C, with Bonferroni-corrected two-tailed t-test or Mann–Whitney rank sum test results. Measurements are expressed as percent standard length. Morphometric codes are provided in the text.

	SL (mm)	TL (mm)	PDLN	PALN	DBSLN	DLN	CLN	ABSLN	ALN	PCLN	PLLN	POAO	PLBS	BDP	CPDP	HDWD	HDLN	INORB	ORBD
FL																			
Mean	21.01	25.86	0.75	0.62	0.09	0.19	0.23	0.14	0.24	0.19	0.07	0.08	0.02	0.17	0.13	0.19	0.27	0.13	0.08
SD	0.85	1.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00
PG																			
Mean	18.95	23.49	0.75	0.63	0.09	0.19	0.24	0.14	0.24	0.19	0.07	0.08	0.02	0.17	0.12	0.20	0.27	0.13	0.08
SD	1.19	1.36	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00
t value	7.02		−1.11		3.52	1.79	−3.35	1.15		−2.08	2.96	0.20		1.28		−1.55	−2.15		−1.88
Mann–Whitney T value				566.50					730.50				861.50		676.00			713.00	
Bonferroni *P* value	<.001*		0.81	1.20	0.002*	0.72	0.03*	1.00	0.70	0.48	0.07	0.84	0.002*	1.00	0.92	1.04	0.39	1.05	0.66

* Significant difference at $P \leq 0.05$.

TABLE 7. Principal components analysis factor loadings and percentage of total variance explained for 11 meristic counts in 11 populations of *Rivulus marmoratus*. Character codes are provided in the text.

	1	2	3
Component loadings			
DR	0.161	0.296	0.542
ANR	0.277	−0.189	0.522
CRBR	0.350	−0.640	−0.230
CRR	−0.103	0.808	−0.008
PCR	0.096	0.393	0.378
PLR	0.089	−0.563	0.100
LSC	0.850	0.231	0.000
TRSC	0.726	0.092	−0.364
PDSC	0.772	0.113	0.142
CPSC	0.627	0.197	−0.443
TOTV	0.429	−0.349	0.552
Percentage of total variance explained by each factor			
	24.33	17.20	12.98

significant (Wilks' lambda = 0.0022; F = 2.34; df = 17,26; $P < 0.001$), and the most similar groups were 1) PG8–PG12; 2) PG8–PG3; and 3) PG3–PG10 and the most dissimilar were 1) VOL–CH; 2) VOL–PG10; and 3) PG10–CH (Fig. 7). Again, individuals within a clone cluster together. The two characters providing the best discrimination were body depth and interorbit distance.

DISCUSSION

Differentiation among wild fish populations.—The use of meristic and morphometric characters

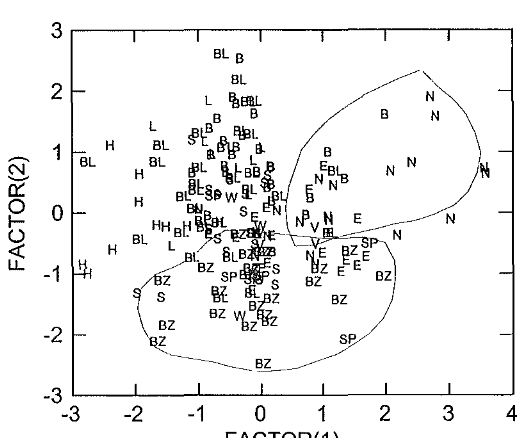

Fig. 2. The PCA factor scores 1 and 2 for meristic counts of individuals from 11 populations of *Rivulus marmoratus*. Population site codes are given in Table 1.

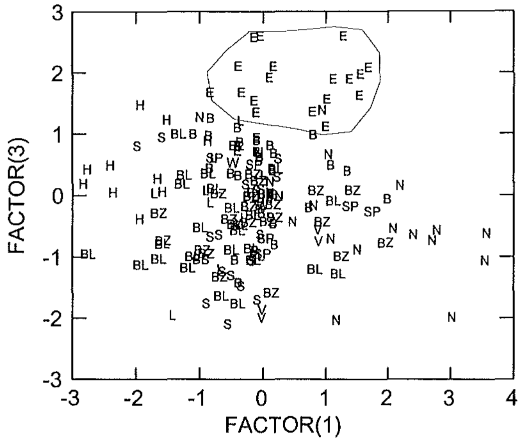

Fig. 3. The PCA factor scores 1 and 3 for meristic counts of individuals from 11 populations of *Rivulus marmoratus*. Population site codes are given in Table 1.

demonstrates that populations of *R. marmoratus* exhibited significant differences throughout 11 populations. Further, because significant differences were noted between offspring of two populations raised at constant temperature, it is likely that some of the differences observed in the wild fish are genetic in origin.

In the wild fish, significant differences were found between all populations for all meristic and morphometric characters (with the exception of pectoral rays), indicating that populations are physically very heterogenous. In addition, standard length is significantly positively correlated with latitude, as is the overall "magnitude" of meristic counts (Fig. 4). The last two findings confirm that Jordan's rule (increase of size or "parts" with increasing lati-

TABLE 8. Principal components analysis factor loadings and percentage of total variance explained for 17 morphometric measurements in 11 populations of *Rivulus marmoratus*. Character codes are provided in the text.

	1	2	3
Component loadings			
PDLN	0.070	0.008	0.002
PALN	0.072	0.006	0.004
DBSLN	0.078	0.011	−0.020
DLN	0.079	0.015	−0.010
CLN	0.060	0.020	−0.010
ABSLN	0.074	0.008	−0.005
ALN	0.077	0.009	−0.002
PCLN	0.066	0.011	−0.006
PLLN	0.102	−0.000	−0.005
POAO	0.067	−0.001	0.034
PLBS	0.112	−0.075	−0.006
BDP	0.086	0.003	0.005
CPDP	0.083	−0.002	0.007
HDWD	0.078	0.008	0.004
HDLN	0.066	0.007	0.004
INORB	0.066	0.007	0.006
ORBD	0.060	0.011	0.007
Percentage of total variance explained by each factor			
	86.78	6.03	1.77

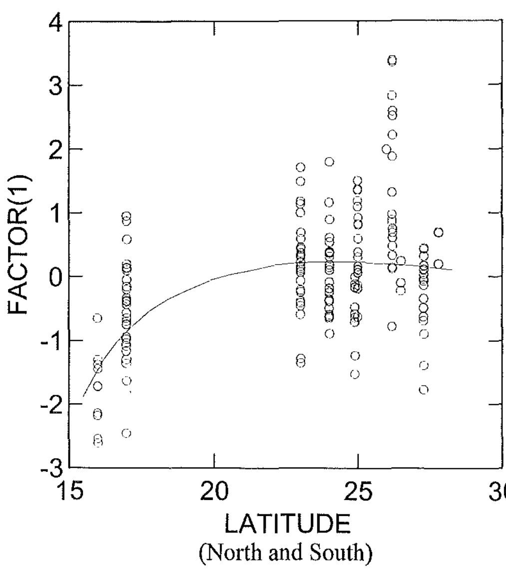

Fig. 4. Linear regression of meristic PCA scores for factor 1 and north–south latitude of collection for 11 populations of *Rivulus marmoratus*. Each circle represents at least one fish (n = 183).

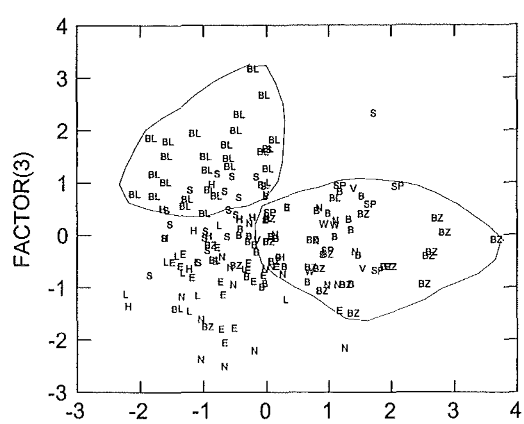

Fig. 5. The PCA factor scores 2 and 3 for morphometric measurements of individuals from 11 populations of *Rivulus marmoratus*. Population site codes are given in Table 1.

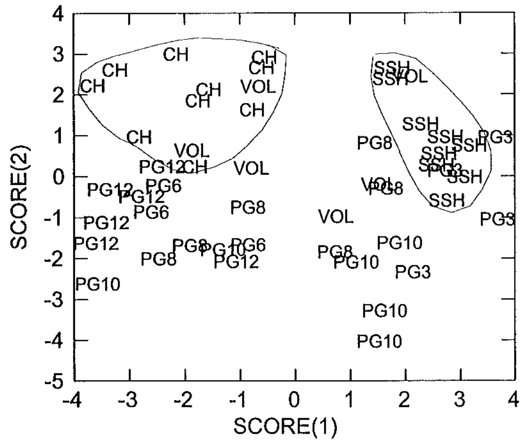

Fig. 6. The DFA canonical scores 1 and 2 for 10 meristic counts from 50 offspring of three Florida (CH, SSH, and VOL) and five Belize (PG3, PG6, PG8, PG10, and PG12) *Rivulus marmoratus*. Population site codes are given in Table 2.

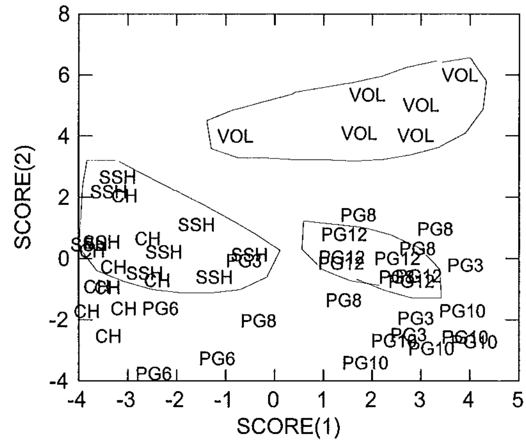

Fig. 7. The DFA canonical scores 1 and 2 for 17 morphometric measurements from 50 offspring of three Florida (CH, SSH, and VOL) and five Belize (PG3, PG6, PG8, PG10, and PG12) *Rivulus marmoratus*. Population site codes are given in Table 2.

tude between populations of the same species) applies to *R. marmoratus*.

The PCA indicates that different populations are often characterized by different trends in various meristic and morphometric parameters. Results of the PCA are summarized in Table 9. This summation shows that *R. marmoratus* from different sites tend to have characteristic patterns in various meristic and morphometric characters.

Brazil–Sao Paulo consistently group together and share with Everglades the common feature of greater ray counts, whereas greater fin lengths dominate in Brazil–Sao Paulo. From these analyses, four populations emerge as distinct from the others: Brazil–Sao Paulo, Everglades, Belize, and Honduras.

Differentiation between hermaphrodite and male fish from Belize.—Huber (1992) determined that there is generally not a strong sexual dimorphism among gonochoristic *Rivulus* species, except for a greater body depth in females, whereas males have larger dorsal and anal fins. However, sexual dichroism is much more pronounced, as is obviously the case with *R. marmoratus*. The finding of greater lengths and bases for dorsal and anal fins and greater lengths for caudal and pectoral fins in male *R. marmoratus* is in keeping with Huber's generalizations. Larger fins in male fish probably are advantageous during courtship displays, during which the larger fins feature the pronounced black banding in the lower edge of the anal, caudal, and tip of dorsal fins and the overall red hues. Although meristic comparisons were not made between hermaphrodite and male *R. marmoratus*, Harrington and Crossman (1976a) raised the gonochoristic *R. cylindraceus* at differing temperatures and found no difference

TABLE 9. Summary of PCA analyses of meristic and morphometric characters in 11 populations of *Rivulus marmoratus*. Meristic and morphometric codes are provided in the text.

Wild fish	
Meristic characters	Morphometric characters
Naples > all scales	Brazil/Sao Paulo > PCLN
Brazil/Sao Paulo > CRBR	Brazil/Sao Paulo > DLN
Brazil/Sao Paulo < CRR	Brazil/Sao Paulo > DBSLN
Brazil/Sao Paulo > PLR	Brazil/Sao Paulo > CLN
Everglades > ANR	Brazil/Sao Paulo < PLBS
Everglades > DR	Brazil/Sao Paulo > ORBD
Everglades > TOTV	Belize > POAO
	Belize < DBSLN

[a] > indicates greater count or measurement; < indicates lesser count or measurement.

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in meristic counts for five characters between males and females.

Discriminant function analysis: Belize–Florida fish reared at 25 C.—The grouping of similarity or dissimilarity in this experiment is of interest because it might logically be expected that clones would align with those most geographically proximate to themselves. This does not appear to be the case in the meristic data, for example, as shown by the Florida clone, SSH, being more similar to PG3 (Belize) than to CH (Florida), and at least two Belize clones are more dissimilar to each other than they are to any of the three Florida clones (Fig. 6). Also, two Florida clones (VOL and CH) were the most dissimilar in morphometrics (Fig. 7).

Perhaps the most remarkable outcome of the DFA analyses of known clonal lineages is that the technique appears to classify individual clones with accuracy (e.g., Figs. 6, 7). Considering that *R. marmoratus* clones have previously been differentiated only at the molecular level by DNA fingerprinting, this finding is noteworthy. It might be expected that some of the Florida clones could be separated because CH and SSH were collected 4 km apart and VOL was collected 105 km north of these. However, all five Belize clones were collected within 75 m of each other (W. P. Davis, pers. comm.).

Several lines of evidence point to *R. marmoratus* as having recently diverged from an ancestral form. Sola et al. (1997) deduced from the nearly uniform karyotypes (both number and morphology) of *R. marmoratus* from four disparate locales [Brazil, Turks and Caicos (Bahamas), Belize, and Florida (four sites)] that the species is "young" and that chromosomal variation has not yet occurred. Murphy (1997) implies that *R. marmoratus* descended directly from *R. caudomarginatus* and that *R. marmoratus* has since spread rapidly north from southeast Brazil. Recent findings on the primitive nature of *R. marmoratus* spermatozoon also suggest a recent origin of the species because *R. marmoratus* spermatozoon resemble more those of externally fertilizing teleosts. External fertilization is regarded as the ancestral condition (Kweon et al., 1998). In another mtDNA study of *R. marmoratus,* Weibel et al. (1999) concur that *R. marmoratus* is one of the more recently diversifying species within *Rivulus,* but they suggest an Antillean origin for the species. Of the three lineages resolved within *R. marmoratus,* the western Caribbean lineage (Florida–Belize) is basically identical and monomorphic for nearly all 16 restriction enzymes examined. Bahamas and Brazil *R. marmoratus* are more similar to each other than they are to either Florida or Belize. The present data do not confirm these molecular associations.

The spate of recent papers on the molecular genetics of the genus *Rivulus* are welcome, yet they raise many intriguing questions. Clearly, these molecular approaches should be pursued, with more sampling over a wider geographic range required. Of particular interest would be collections from the vast area of southern Brazil to northern South America, from which there are no extant collections. Between these types of studies and further work on the meristics and morphology of different populations, some of the questions remaining about the origin and dispersal of this unusual species may be answered.

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Literature Cited

Davis, W. P. 1986. The role of *Rivulus marmoratus* in research on aquatic pollutants. J. Am. Killifish Assoc. 19:70–80.

———, D. S. Taylor, and B. J. Turner. 1990. Field observations on the ecology and habits of the mangrove rivulus (*Rivulus marmoratus*) in Belize and Florida. Ichthyol. Explor. Freshw. 1:123–134.

Ehlinger, T. J., and D. S. Wilson. 1988. Complex foraging polymorphism in bluegill sunfish. Proc. Natl. Acad. Sci. 85:1878–1882.

Fowler, J. A. 1970. Control of vertebral number in teleosts—an embryological problem. Q. Rev. Biol. 45:148–167.

Harrington, R. W., Jr. 1961. Oviparous hermaphroditic fish with internal fertilization. Science 134: 1749–1750.

———. 1967. Environmentally controlled induction of primary male gonochorists from eggs of the self-fertilizing hermaphroditic fish, *Rivulus marmoratus.* Biol. Bull. 132:174–199.

———, and R. A. Crossman. 1976a. Effects of temperature and sex genotype on meristic counts of the gonochoristic cyprinodontid fish *Rivulus cylindraceus* Poey. Can. J. Zool. 54:245–254.

———, and ———. 1976b. Temperature induced meristic variation among three homozygous genotypes (clones) of the self-fertilizing fish *Rivulus marmoratus*. Can. J. Zool. 54:1143–1155.

———, AND K. D. KALLMAN. 1968. The homozygosity of clones of the self-fertilizing hermaphroditic fish *Rivulus marmoratus* (Cyprinodontidae, Atheriniformes). Am. Nat. 102:337–343.

———, AND L. R. RIVAS. 1958. The discovery in Florida of the cyprinodontid fish *Rivulus marmoratus*, with a redescription and ecological notes. Copeia 1958:125–130.

HOEDEMAN, J. J. 1958. The frontal scalation pattern in some groups of toothcarps (Pisces: Cyprinodontiformes). Bull. Aquat. Biol. 1:23–28.

HUBBS, C. L., AND K. F. LAGLER. 1958. Fishes of the Great Lakes Region. Univ. of Michigan Press, Ann Arbor, MI.

HUBER, J. H. 1992. Review of *Rivulus*, ecobiogeography, relationships. Societe Francaise d'Ichtyologie, Paris.

KALLMAN, K. D., AND R. W. HARRINGTON JR. 1964. Evidence for the existence of homozygous clones in the self-fertilizing hermaphroditic teleost *Rivulus marmoratus* Poey. Biol. Bull. 126:101–114.

KOENIG, C. C., AND M. P. CHASAR. 1984. The usefulness of the hermaphroditic marine fish, *Rivulus marmoratus*, in carcinogenicity testing. Natl. Cancer Inst. Monogr. 65:15–33.

KRISTENSEN, I. 1970. Competition in three cyprinodont fish species in the Netherlands Antilles. Stud. Fauna Curacao Other Caribb. Isl. 119:82–101.

KWEON, H.-S., E.-H. PARK, AND N. PETERS. 1998. Spermatozoon ultrastructure in the internally self-fertilizing hermaphroditic teleost, *Rivulus marmoratus* (Cyprinodontiformes; Rivulidae). Copeia 1998: 1101–1106.

LINDSEY, C. C., AND R. W. HARRINGTON JR. 1972. Extreme vertebral variation induced by temperature in a homozygous clone of the self-fertilizing cyprinodontid fish *Rivulus marmoratus*. Can. J. Zool. 50: 733–744.

LUBINSKI, B. A., W. P. DAVIS, D. S. TAYLOR, AND B. J. TURNER. 1995. Outcrossing in a natural population of self-fertilizing hermaphroditic fish. J. Hered. 86: 469–473.

MURPHY, W. J. 1997. Molecular systematics and biogeography of fishes of the suborder Aplocheiloidei (Atherinomorpha, Cyprinodontiformes). Unpubl. Ph.D. diss., Univ. of Tulsa, Tulsa, OK.

———, AND G. E. COLLIER. 1996. Phylogenetic relationships within the aplocheiloid fish genus *Rivulus* (Cyprinodontiformes, Rivulidae): implications for Caribbean and Central American biogeography. Mol. Biol. Evol. 13:642–649.

RIVAS, L. R. 1944. Contributions to the study of the poeciliid fishes of Cuba. I. Descriptions of six new species of the subfamily Gambusiinae. Proc. N. Engl. Zool. Club 23:41–53.

———. 1945. The discovery and redescription of the types of *Rivulus marmoratus* Poey, a cyprinodontid fish from Cuba. J. Wash. Acad. Sci. 35:95–97.

SHEPHERD, G. 1991. Meristic and morphometric variation in black sea bass north of Cape Hatteras, North Carolina. N. Am. J. Fish. Manag. 11:139–148.

SOLA, L., M. MARZOVILLO, A. R. ROSSI, E. GORNUNG, S. BRESSANELLO, AND B. J. TURNER. 1997. Cytogenetic analysis of a self-fertilizing fish, *Rivulus marmoratus*: remarkable chromosomal constancy over a vast geographic range. Genome 40:945–949.

SWAIN, D. P., AND C. C. LINDSEY. 1986a. Influence of reproductive history of parents on meristic variation in the offspring in the cyprinodont fish *Rivulus marmoratus*. Can. J. Zool. 64:1456–1459.

———, AND ———. 1986b. Meristic variation in a clone of the cyprinodont fish *Rivulus marmoratus* related to temperature history of the parents and of the embryos. Can. J. Zool. 64:1444–1455.

TAPHORN, D. C. 1980. First record of *Rivulus marmoratus* Poey, 1880 from the South American continent (Pisces: Cyprinondontidae). Zool. Meded. 55:127–129.

TAYLOR, D. S. 1988. Observations on the ecology of the killifish *Rivulus marmoratus* Poey (Cyprinodontidae) in an infrequently flooded mangrove swamp. Northeast Gulf Sci. 10:63–68.

———. 1990. Adaptive specializations of the cyprinodont fish *Rivulus marmoratus*. Fla. Sci. 53:239–248.

———. 1993. Notes on the impact of the December 1989 freeze on local populations of *Rivulus marmoratus* in Florida, with additional distribution records in the state. Fla. Sci. 56:129–134.

———. 2001. Physical variability and fluctuating asymmetry in heterozygous and homozygous populations of *Rivulus marmoratus*. Can. J. Zool. 79: 766–778.

———, M. T. FISHER, AND B. J. TURNER. 2001. Homozygosity and heterozygosity in three populations of *Rivulus marmoratus*. Environ. Biol. Fish. 61: 455–459.

TURNER, B. J., W. P. DAVIS, AND D. S. TAYLOR. 1992a. Abundant males in populations of selfing hermaphrodite fish, *Rivulus marmoratus*, from some Belize cays. J. Fish Biol. 40:307–310.

———, J. F. ELDER JR., T. F. LAUGHLIN, W. P. DAVIS, AND D. S. TAYLOR. 1992b. Extreme clonal diversity and divergence in populations of a selfing hermaphroditic fish. Proc. Natl. Acad. Sci. U.S.A. 89: 10643–10647.

WEIBEL, A. C., T. E. DOWLING, AND B. J. TURNER. 1999. Evidence that an outcrossing population is a derived lineage in a hermaphroditic fish (*Rivulus marmoratus*). Evolution 53:1217–1225.

WILLIAMS, B. K., AND K. TITUS. 1988. Assessment of sampling stability in ecological applications of discriminant analysis. Ecology 69:1275–1285.

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