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**ELECTROPHORETIC COMPARISON OF *Cyprinodon variegatus*
LACÉPÈDE AND *Cyprinodon hubbsi* CARR,
WITH COMMENTS ON THE GENUS *Cyprinodon*
(Atheriniformes: Cyprinodontidae)**

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Abstract: Five populations of *Cyprinodon hubbsi* were electrophoretically compared to 12 populations from Florida of *C. variegatus*. Our data support Johnson and Snelson's (1978) placing of *C. hubbsi* in synonymy with *C. variegatus*. Present day populations of the nominal *C. hubbsi* possibly arose from 3 different founding populations: one derived from a Gulf coast *C. variegatus* population, another derived from a Florida east coast *C. variegatus* population, and a third from which the present day Lake Dora population of *C. hubbsi* is derived, origin uncertain. The large amount of morphological variation in this genus is also partially reflected electrophoretically, and the populations sampled fall into discrete groups.

A remarkable array of pupfish species, genus *Cyprinodon*, occur in the United States, Mexico, the Bahama Islands and on some Caribbean Islands. Most species, some as yet undescribed, are geographically restricted, allopatric populations. Only *Cyprinodon variegatus* has a wide distribution, and a number of nominal allopatric species are seemingly related to it, constituting a "*variegatus* complex" within the genus. For details of the "*variegatus* complex" and for other pupfish species see Turner and Liu, 1977; Miller, 1948, 1968, 1976, 1981; Echelle and Miller, 1974; Echelle and Echelle, 1978; Echelle, 1975; Turner, 1973, 1974; Miller and

Echelle, 1975; Liu, 1969; Smith and Miller, 1980; Humphries and Miller, 1981; Soltz and Hirshfield, 1981.

Cyprinodon variegatus Lacépède, the sheepshead minnow, ranges from Massachusetts southward along the Atlantic coast through the Florida Keys, throughout the Gulf of Mexico to northeastern Mexico, and disjunctly in Yucatan (as *C. v. artifrons*, Hubbs, 1936). Additional populations in the Bahamas and on Caribbean Islands have been considered as subspecies of *C. variegatus* or as related species within the "*variegatus* complex" (Turner and Liu, 1977). Hubbs (1936) delineated northern and southern

subspecies, *C. v. ovinus*, Massachusetts to North Carolina, and *C. v. variegatus*, North Carolina to northeastern Mexico, as well as *C. v. artifrons* from Yucatan. Earlier, Jordan (1884) had placed populations in Cuba and the Florida Keys in the subspecies *C. v. riverendi*. No published data support these subspecific allocations, nor are there any published definitive studies, other than original descriptions earlier in the century, of outlying allopatric populations in the complex in the Bahamas, Yucatan, and the Caribbean. Texas and northern Mexico populations of the “*variegatus* complex” have been delineated by Echelle and Echelle (1978), Echelle and Miller (1974) and Miller and Echelle (1975), but electrophoretic techniques have not been employed. In addition, *Cyprinodon* populations in several central Florida freshwater lakes have either been designated as *C. hubbsi* or *C. v. hubbsi* by various authors (Carr, 1936; Johnson and Snelson, 1978; Humphries and Miller, 1981).

Cyprinodon variegatus and its related forms are euryhaline (Simpson and Gunter, 1956; Martin, 1968). We have collected *C. variegatus* on Big Pine Key, Florida, from salinities of 5 ppt and 63 ppt on the same day at localities approximately 2 km apart. We also collected the species at the northern end of Key Largo in a hypersaline lagoon at 81 ppt. Nearby, on the southern end of the Florida peninsula from Lake Okeechobee southward through the Everglades, *C. variegatus* occurs in fresh or brackish waters (Ager, 1971; Kushlan and Lodge, 1974). Miller (1948) suggested a positive correlation between meristic features and salinity, but did not apply rigorous statistical tests to his data, for desert pupfishes in the western United States. Our electrophoretic data, and the meristic data of Johnson and Snelson (1978), do not indicate such a correlation for Florida *Cyprinodon*. This is an important point since the nominal *C. hubbsi*,

considered in this paper, is a freshwater form.

Cyprinodon hubbsi Carr is known only from a few central Florida lakes: Lake Eustis (type locality; Carr, 1936), Lakes Dora, Griffin, Harris, Yale, Weir and Silver (Relyea, 1975; Johnson and Snelson, 1978). All of these lakes are interconnected by canals and ditches and are drained by the Ocklawaha River, a western tributary of the St. Johns River. However, the canals are often blocked by spillways, are steep sided and generally do not have pupfish habitat and do not, as far as known, support pupfish populations. *Cyprinodon hubbsi* prefers open sandy areas, often with a light silt cover, in very shallow littoral zones often including nearby patches of emergent vegetation. We have also found that *C. hubbsi* (and *C. variegatus*) will aggregate on hard, algal covered substrates of boat ramps. Although *Cyprinodon variegatus* occurs in some areas of the St. Johns River, the species has not been collected, despite intensive surveys, from the Ocklawaha.

Carr (1936) delineated *C. hubbsi* from *C. variegatus* on the basis of several morphometric and meristic features. Since that publication, Johnson and Snelson's (1978) brief report using morphological data, based in part on Johnson's unpublished Master's thesis, Univ. of Central Florida, remains the only recent taxonomic analysis of *C. hubbsi* and its relationship to *C. variegatus*. Johnson and Snelson's (1978) report is important as it includes several populations of the nominal *C. hubbsi* and demonstrates more of the existing morphological variation within that form than Carr's (1936) original description. Johnson and Snelson (1978) concluded that *C. hubbsi* should be relegated to the synonymy of *C. variegatus*.

We present electrophoretic data for Lakes Eustis, Dora, Weir, Harris and Griffin, all “*hubbsi*” lakes. Lake Weir is the

most isolated geographically of these.

The purpose of this paper is to present data from our investigations on two aspects of the problem of speciation in the genus *Cyprinodon*:

1. genetic variability in *Cyprinodon variegatus* in Florida.
2. the relationship of nominal *Cyprinodon hubbsi* populations to one another and to *C. variegatus*.

Our analysis should provide insights into speciation in the genus *Cyprinodon* and establish a foundation for examination by electrophoretic methods of allopatric populations in Texas freshwaters, northern Mexico, Yucatan, the Bahamas and on Caribbean Islands.

MATERIALS AND METHODS

Collections were made with a 4.6 meter (7mm mesh) seine. The fishes were placed in a Zip-Loc bag, covered with the water in which they were collected, and

placed on a block of dry ice until returned to the laboratory. In the field, salinity was recorded with an American Optical Instruments refractometer. Twenty specimens were used for electrophoresis from each locality (Figure 1). All collections were made in May, 1981 except where noted.

Cyprinodon variegatus: Florida: (population number 1) Brevard Co., Cocoa Beach, Dec. 1980, salinity not recorded; (2) Indian River Co., Long Point Co. Park, 16 ‰; (3) Indian River Co., Sebastian Inlet, 36 ‰; (4) St. Johns Co., Anastasia St. Park, 32 ‰; (5) Monroe Co., Key Largo, 81 ‰; (6) Monroe Co., Grassy Key, 60 ‰; (7) Monroe Co., Big Pine Key, 63 ‰; (8) Hillsborough Co., Alafia River @ Rte. 41, 34 ‰; (9) Manatee Co., Causeway to Anna Maria Beach (Rte. 64), 36 ‰; (10) Wakulla Co., pond at St. Marks Lighthouse, 16 ‰; (11) Wakulla Co., Gulf coast at Panacea, 37 ‰; (12) Wakulla Co., Panacea, tidal creek, Dec. 1980, salinity not recorded.

Cyprinodon hubbsi: Florida: (13) Lake Co., Lake Harris at Leesburg; (14) Lake Co., Lake Dora at Mt. Dora; (15) Marion Co., Lake Weir at Oklawaha; (16) Lake Co., Lake Griffin at Coca Cola Park, on east side of lake; (17) Lake Co., Lake Eustis at Eustis. Salinity 0‰ at all *C. hubbsi* localities.

To obtain protein samples for electrophoresis, individual fish were homogenized in an equal volume of chilled distilled water; the slurry that resulted was centrifuged at 25,000g at 4°C for 60 min. The supernatant of water soluble proteins was decanted and stored at 4°C overnight, a maximum of 18 hrs. prior to electrophoretic separations.

The 20 loci coding for proteins surveyed in this study were: nonenzymatic proteins (*Gp-1*, 2, 3, 4, 5); esterases (*Est-1*, 2, 3, 4); glucosephosphate isomerases (*Gpi-A*, *B*); glycerol-3-phosphate dehydrogenase (*G-3-pdh-A*); lactate dehydrogenases (*Ldh-A*, *B*, *C*); malate

Figure 1. Collection localities and numbers of populations of *Cyprinodon* examined in this study.

dehydrogenases (NAD dependent) (*S-Mdh-A, B*); phosphoglucumutase (*Pgm-A*); superoxide dismutase (*Sod-A*); and xanthine dehydrogenase (*Xdh-A*).

Techniques of starch gel electrophoresis were similar to those described by Brewer (1970) and Selander *et al.* (1971) with the following modifications: *Gp*, *Est*, *Gpi*, and *Ldh* were resolved on the LiOH discontinuous ion system described by Selander *et al.* (1971), and *G-3-pdh*, *Mdh*, *Pgm*, *Sod* and *Xdh* were surveyed on the tris-citrate-EDTA pH 7.1 ion system described by Ayala *et al.* (1972). All gels were 12.5% starch (Electrostarch, Lot 307, Otto Hiller Electrostarch Co., Madison, Wisconsin.).

The locus nomenclature system follows Fisher *et al.* (1980) and Crabtree and Buth (1981). When electromorph (allelic) variation occurred, the electromorph with the greatest anodal migration was called *a*, the next *b*, and so on. Electrophoretic data were summarized with Nei's (1972) standard genetic distance statistic, *D*.

RESULTS AND DISCUSSION

Of the 20 loci coding for proteins

surveyed in this study, four (*Ldh-C*, *S-Mdh-A*, *Sod-A*, *Xdh-A*) were fixed for the same electromorphs in all samples. Electromorph frequency variation was observed for the remaining 16 loci (Table 1). Genetic distance was calculated between sample populations (Table 2) and a dendrogram (Fig. 2) was constructed from these distance matrix data. In Fig. 2 it is apparent that there are three major population clusters containing populations united at genetic distances of 0.05 or less. These three clusters are: the east coast *C. variegatus* populations plus populations of *C. hubbsi* from Lakes Harris, Griffin and Eustis; the Florida Keys populations (nominally *C. v. riverendii*); and four of the five Gulf coast *C. variegatus* populations, including a population of *C. hubbsi* from Lake Weir. These clusters reflect a sharing of otherwise uncommon electromorphs at high frequencies at one or a few loci among the populations of each cluster (Table 1). The east coast cluster possesses the otherwise uncommon *Est-4 a* electromorph at frequencies of 0.90 or greater. The Florida Keys populations are unique at *Gp-2*, where they possess the *c* electromorph at frequencies of 0.77 or more, whereas the frequency of that elec-

Figure 2. Dendrogram (UWPGA) representing relationships as determined by electromorph frequencies for populations examined in the genus *Cyprinodon* (see Materials and Methods for localities of numbered populations).

Table 1. Electromorph frequencies for 16 polymorphic loci. See Material Examined or Figure 1 for localities of numbered populations. Symbols: h = *C. hubbsi*, v = *C. variegatus*.

Locus	Population																	
	1v	2v	3v	4v	5v	6v	7v	8v	9v	10v	11v	12v	13h	14h	15h	16h	17h	
Est-1	a											0.02						
	b											0.93						
	c			0.02			0.18			0.02								
	d	0.25	0.19	0.42	0.25	0.80	0.62	0.67	1.0	0.45	0.85	0.47	0.05	0.42	0.84	0.15	0.05	0.53
	e	0.75	0.77	0.56	0.73	0.20	0.38	0.15		0.55	0.13	0.48		0.58	0.16	0.84	0.95	0.47
	f		0.04		0.02							0.05				0.01		
Est-2	a				0.02				0.05			0.52						
	b				0.08				0.10			0.38						
	c									0.10	0.40	0.10	0.02	0.03				
	d	0.73	0.84	0.94	0.50	0.35	0.75	1.0	0.88	0.45	0.27	0.47		0.98	0.94	1.0	0.98	0.93
	e	0.27	0.16	0.06	0.50	0.55	0.23		0.10	0.40	0.40	0.13			0.03		0.02	0.05
	f					0.02		0.02			0.23							0.02
Est-3	a											0.07						
	b											0.88						
	c			0.02	0.10			0.02					0.02	0.13				
	d	0.43	0.79	0.14	0.20					0.10	0.10	0.02		0.18	0.26		0.55	0.60
	e	0.57	0.21	0.84	0.70	0.92	0.85	0.98	0.93	0.88	0.78	0.58	0.05	0.77	0.39	0.90	0.45	0.35
	f					0.08	0.15		0.07	0.02	0.12	0.22		0.03	0.22	0.10		0.05
	g											0.18						
Est-4	a	0.95	1.0	0.98	0.93	0.10	0.45	0.40		0.08	0.05	0.02	0.13	0.90	0.33		0.95	0.98
	b	0.05		0.02	0.07	0.90		0.08		0.02					0.67	0.10	0.05	0.02
	c							0.02		0.03								
	d						0.55	0.50	1.0	0.87	0.95	0.98	0.87	0.10		0.90		
Gp-1	a	1.0	0.93	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	b		0.07															
Gp-2	a																	0.02
	b	1.0	0.90	1.0	0.70	0.23	0.05	0.13	1.0	0.75	1.0	1.0	1.0	0.95	0.89	0.95	1.0	0.83
	c		0.10		0.30	0.77	0.95	0.87		0.25				0.05	0.11	0.05		0.15
Gp-3	a		0.02					0.10						0.01			0.05	
	b	1.0	0.98	1.0	0.75	1.0	1.0	0.90	0.78	0.95	1.0	0.95	1.0	0.98	1.0	1.0	0.95	1.0
	c				0.25				0.22	0.05		0.05		0.01				
Gp-4	a	0.13										0.10						
	b	0.07											0.02					
	c	0.80	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.90	0.98	1.0	1.0	1.0	1.0	1.0
Gp-5	a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.95	1.0	1.0	1.0	1.0	1.0	1.0
	b											0.02						
	c											0.03						
G-3-pdh-A	a	0.98	0.98	1.0	0.98	1.0	1.0	0.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	b	0.02	0.02		0.02			0.02										
Gpi-A	a	0.05	0.04	0.06		0.18	0.33	0.25	0.23	0.15	0.10	0.23	0.28	0.01		0.02		
	b	0.93	0.96	0.94	1.0	0.82	0.67	0.75	0.77	0.85	0.88	0.77	0.72	0.98	1.0	0.98	1.0	1.0
	c	0.02									0.02		0.01					
Gpi-B	a								0.02	0.02								
	b	1.0	1.0	0.98	1.0	1.0	1.0	0.98	1.0	0.98	0.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	c			0.02				0.02										
Ldh-A	a							0.10									0.15	
	b	1.0	1.0	1.0	1.0	1.0	1.0	0.90	1.0	1.0	1.0	1.0	1.0			1.0	0.85	1.0
	c													1.0				
Ldh-B	a					0.02		0.02	0.05									
	b	1.0	1.0	0.98	1.0	1.0	0.98	0.93	0.98	0.95	1.0	1.0	0.90	1.0	1.0	1.0	0.98	0.95
	c			0.02				0.07					0.10				0.02	0.05
S-Mdh-B	a										0.02		0.03	0.09		0.13	0.18	
	b	1.0	1.0	1.0	0.93	1.0	1.0	1.0	0.97	1.0	0.98	1.0	0.97	0.91	0.98	0.87	0.82	
	c				0.07				0.03						0.02			
Pgm-A	a						0.02		0.02	0.02								
	b	0.98	1.0	1.0	1.0	1.0	1.0	0.98	1.0	0.98	0.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	c	0.02																

tromorph is 0.30 or less in all other *Cyprinodon* populations examined here. The Gulf coast cluster is not notably unique at any locus. It differs from the east coast population cluster in having the *Est-4a* electromorph only at low frequencies (0.08 or less), and differs from the Florida Keys populations in having the *Gp-2 c* electromorph at a frequency of 0.22 or less. The Lake Weir *C. hubbsi* population clusters with the Gulf coast cluster largely because it possesses the *Est-4 d* electromorph at a frequency of 0.90. This electromorph is rare or absent in other *C. hubbsi* populations, and occurs in high frequency (0.87 or greater) in the cluster of Gulf coast populations. In addition to the three major clusters, two populations are distinct (Fig. 2); the Lake Dora population (14) of *C. hubbsi* and a population (12) of *C. variegatus* from near Panacea, Florida in the northern Gulf of Mexico. The latter population did not cluster with other Gulf coast populations, but another population (11), only 5 km away, did. The Lake Dora population is unique and fixed for the *c* electromorph at *Ldh-A*. This electromorph was not found in any other *Cyprinodon*

population examined in this study. This population also possessed the *Est-4 b* electromorph at a frequency of 0.67. This electromorph is rare (frequencies of 0.10 or less) in other *C. hubbsi* populations. The Panacea population (12) of *C. variegatus* possessed unique electromorphs at high frequencies at *Est-1 (b)*, and *Est-3 (b)*. These electromorphs were not found in other *Cyprinodon* populations studied, including the other Panacea population only 5 km distant. In addition, this unusual Panacea population possessed the otherwise rare electromorphs *Est-2 a* and *b* at moderate frequencies (0.52 and 0.38, respectively).

The data summarized in the dendrogram and the genetic distance matrix allow several conclusions concerning *C. hubbsi*, and *Cyprinodon* in general. One observation is that although the populations sampled fall into discrete groups (clusters), the genetic distance between any two populations examined between clusters is small, maximally 0.20 (Table 2). Our data do not support the existence of more than one species of *Cyprinodon* in Florida among the 17 populations sampled. The large amount of morphological

Table 2. Standard genetic distance between populations of *Cyprinodon*. (Population numbers as in Table 1; abbreviations are v = *Cyprinodon variegatus*, h = *Cyprinodon hubbsi*.)

	Population															
	1v	2v	3v	4v	5v	6v	7v	8v	9v	10v	11v	12v	13h	14h	15h	16h
2v	0.011															
3v	0.010	0.026														
4v	0.016	0.030	0.022													
5v	0.113	0.141	0.104	0.079												
6v	0.091	0.105	0.076	0.065	0.048											
7v	0.097	0.112	0.071	0.076	0.054	0.009										
8v	0.104	0.132	0.078	0.106	0.106	0.076	0.063									
9v	0.062	0.086	0.059	0.052	0.069	0.044	0.053	0.032								
10v	0.086	0.114	0.078	0.084	0.088	0.076	0.081	0.020	0.017							
11v	0.074	0.094	0.070	0.079	0.109	0.076	0.082	0.030	0.017	0.018						
12v	0.159	0.172	0.167	0.169	0.202	0.179	0.189	0.137	0.109	0.106	0.094					
13h	0.010	0.024	0.001	0.022	0.103	0.070	0.064	0.072	0.053	0.073	0.061	0.159				
14h	0.112	0.120	0.100	0.124	0.120	0.146	0.134	0.117	0.131	0.127	0.130	0.227	0.096			
15h	0.062	0.083	0.053	0.071	0.117	0.075	0.073	0.045	0.021	0.049	0.025	0.134	0.045	0.130		
16h	0.011	0.009	0.019	0.030	0.150	0.113	0.114	0.128	0.084	0.120	0.091	0.181	0.016	0.114	0.064	
17h	0.014	0.010	0.016	0.029	0.112	0.081	0.079	0.098	0.076	0.091	0.084	0.169	0.013	0.093	0.078	0.016

variation reported for *C. variegatus* by others (Johnson and Snelson, 1978, based on Johnson's Master Thesis, Univ. of Central Florida; Hubbs, 1936) is partially reflected in the electrophoretic data presented here. We support Johnson and Snelson's (1978) conclusion that *C. hubbsi* is a synonym of *C. variegatus*.

Another observation is that the ancestors of the nominal *C. hubbsi* possibly invaded the central Florida lakes as many as three times, and that these invasions came from different founding populations: a Gulf coast population that founded the Lake Weir population, an east coast founding population (ancestors of Lakes Harris, Griffin, and Eustis populations) and a Lake Dora founding population, origin uncertain. The Lake Dora population, unique for the *c* electromorph at *Ldh-A*, is most similar to population 3 (Sebastian Inlet, $D = 0.10$) and population 13 (Lake Harris, $D = 0.096$). The possibility of founder effects and genetic drift, however, clouds our interpretation of the origin of *C. hubbsi*, and we offer the above speculation as a basis for continued investigations.

Population 12 from near Panacea, Florida on Florida's northern Gulf coast is especially puzzling. It is quite distinct, as noted earlier, from a nearby population from which there are no obvious geographic or ecological barriers in the extensive coastal marsh of that area. Population 12 was collected in winter (December) and the fish were spawning, along with large numbers of *Fundulus grandis*. The other Panacea collection was made in summer and consisted of non-spawning individuals in a tidal marsh with similar ecological conditions, as far as is known. We can only speculate that either 1) we sampled an inland spawning aggregation of a population of *Cyprinodon variegatus* which normally inhabits another locality, e.g. the offshore barrier islands which oc-

cur in the area, or 2) we have demonstrated some seasonal variation in expression of certain genes. Both possibilities are worthy of further investigation. In any case, these data appear to reflect two different populations in the Panacea area of one highly variable (polytypic) species. The existence of many independent local populations, possibly with little interbreeding, may be characteristic of the genus *Cyprinodon*, a point supported by Darling's electrophoretic data (Unpublished Ph.D. dissertation, Yale University, 1976). This may explain both the large interpopulation variation, morphological and electrophoretic, and the large number of allopatric species which have evolved in the *C. variegatus* complex.

Our electrophoretic data also support Stevenson's (1981) concept that the nominal, mostly allopatric, populations of *Cyprinodon* best fit a semispecies concept. The subgroupings within the genus, i.e. "*variegatus* complex", constitute superspecies composed of allopatric semispecies populations. Stevenson (1981) also demonstrated karyotic conservatism within the genus. All species studied to date have $2N = 48$ chromosomes and there is little chromosome morphological variability. Liu (1969) and Turner and Liu (1977) showed that there were no barriers to hybridization between allopatric *Cyprinodon* "species" in laboratory "forced" or "no choice" mating situations despite natural ethological differences. Turner (1974) also demonstrated that there was little genetic variability between allopatric Death Valley pupfish populations ("*nevadensis* complex") despite considerable morphological divergence (also see Miller, 1948, 1981; Soltz and Hirshfield, 1981). Implied is extreme genetic conservatism but outward morphological and behavioral divergence, although this divergence may be less than intrageneric differences seen in other cyprinodontid

groups such as *Fundulus*. Our data for Florida *Cyprinodon* populations reveal somewhat greater genetic variability than Turner's (1974) for western pupfish, but do not support the recognition of more than one species. *Cyprinodon variegatus* should be viewed as a polytypic species including the various populations of the nominal *C. hubbsi*.

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