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Systematics of the Key Silverside, *Menidia conchorum*, with Comments on other *Menidia* species (Pisces: Atherinidae)

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Abstract

Populations of *Menidia conchorum* are electrophoretically and meristically compared to *M. beryllina, M. peninsulae* and *M. colei*. The ecological status of *M. conchorum* is also discussed.

Meristically, *Menidia conchorum* and *M. peninsulae* differ to an extent that could by traditional taxonomic analysis be considered distinct species. Electrophoretically, *M. conchorum* does differ from *M. beryllina* and *M. colei*, but not from *M. peninsulae*. We feel that in *Menidia*, the electrophoretic data are more reliable than the meristic data because of environmental influences on meristics. Therefore, we conclude that *M. conchorum* and *M. peninsulae* are conspecific. The name *Menidia peninsulae* has nomenclatural priority. Johnson's (1975) distinction between *M. beryllina* and *M. peninsulae* is also supported.

Introduction

The genus *Menidia* as currently recognized contains eight or nine species (Robbins, 1969; Johnson, 1975; Echelle and Mosier, 1981,1982; Chernoff et al., 1981): *Menidia menidia* (L) from Newfoundland to northeastern Florida; *M. beryllina* (Cope) from Massachusetts to Vera Cruz, Mexico; *M. audens* Hay, Mississippi Valley, considered conspecific with *M. beryllina* by Chernoff et al. (1981); *M. extensa* Hubbs and Raney, restricted to Lake Waccamaw, North Carolina; *M. peninsulae* (Goode and Bean), Atlantic coast from northeastern Florida to Ft. Pierce, Florida, and on the Gulf of Mexico coast from Marco Island, Florida to near Brownsville, Texas (eastern and western Gulf populations may be allopatric, Chernoff et al., 1981); *M. clarkhubbsi* Echelle and Mosier, an all female species from the Texas Gulf coast; *M. conchorum* Hildebrand and Ginsburg, Florida Keys; and *M. colei* Hubbs, Yucatan, Mexico.

Menidia colei was placed with *M. conchorum* in a new genus, *Menidiella*, by Schultz (1948). This action has not been generally followed. Miller (1966, 1976) allocated *M. colei* and *M. conchorum* to the genus *Menidia* even though no data were presented, nor other studies cited, to support such a decision. We follow Miller (1966,1976), although we feel that a critical generic level study is in order. Since *M. colei* and *M. conchorum* were originally considered by Schultz

(1948) to be closely related, we include an examination of the relationships of the two forms in this paper, in addition to comparisions of *M. conchorum* to *M. beryllina* and *M. peninsulae*. We do not treat further the generic level taxonomic problem.

The Key Silverside, *Menidia conchorum* Hildebrand and Ginsburg (1927), is distinguished from other *Menidia* by its low anal fin ray number compared to all species except *M. colei* and by its small size. Gilbert (1978) noted that the Key Silverside could also be distinguished by its lower number of lateral and predorsal scales. Only *M. colei* has lower numbers for these traits (Tables 2-5). Air bladder morphology has been used to distinguish *M. beryllina* from *M. peninsulae* (Echelle and Mosier, 1981), and *M. conchorum* resembles *M. peninsulae* in this trait. However, use of this trait for identification has been controversial and we refer the reader to Echelle and Mosier (1981, 1982), Edwards et al. (1978) and Chernoff et al. (1981). We feel that the trait can be used for field identification, and thus follow Echelle and Mosier (1981). In addition, Chernoff et al. (1981) showed that in *M. peninsulae* the anal fin originates under the last precaudal or first caudal vertebra, while in *M. beryllina* it originates one or two vertebrae farther forward. *Menidia conchorum* again resembles *M. peninsulae* in this characteristic.

Based on its limited geographic distribution in the lower Florida Keys, *M. conchorum* was designated as an endangered species (Gilbert, 1978). Continued investigation has resulted in the gradual extension of the known range of *M. conchorum*. Hildebrand and Ginsburg (1927) and Robbins (1969) reported *M. conchorum* only from Boca Chica (near Key West in the lower Florida Keys). One of us (KR) collected the species at Big Pine Key in 1965. Johnson (1975) and Miller (1976) considered *M. conchorum* a rare species, but Gilbert (1978) reported Key Silversides to be wide-spread in the lower Florida Keys. Field collections for the present study revealed seemingly large populations of *M. conchorum* in the middle Florida Keys (Long and Grassy Keys) as well, narrowing the gap in the presumed allopatric distribution of *M. conchorum* with respect to congeners *M. beryllina* and *M. peninsulae*.

In the present study, we define the relationships of the nominal *M. conchorum* by use of both morphological and biochemical characters to other *Menidia* species, and examine variation within the nominal *M. conchorum*. This has necessitated examination of meristic, morphometric and electrophoretic traits for *M. conchorum, M. peninsulae. M. colei, M. beryllina* and *M. menidia*. The latter is only marginally treated as it is quite distinct and geographically far removed from *M. conchorum*. The lacustrine *M. extensa* and the nominal *M. audens* are not considered. *Menidia clarkhubbsi*, of presumed hybrid origin (see Echelle and Mosier, 1981, 1982) is likewise not considered. We include biochemical data for *M. colei* only for comparative purposes to *M. conchorum*, not for analysis in itself, as Echelle will report more extensive data for that species.

Materials and Methods

Materials.—Between June, 1977 and December, 1979, 840 individuals from 21 localities (see locality data and Figure 1) were collected. Collections of *Menidia* were made with a seine or by dipnetting from a shallow draft boat at night. *Menidia* species were separated by taxon when more than one taxon occurred at a locality, and then each species collec-

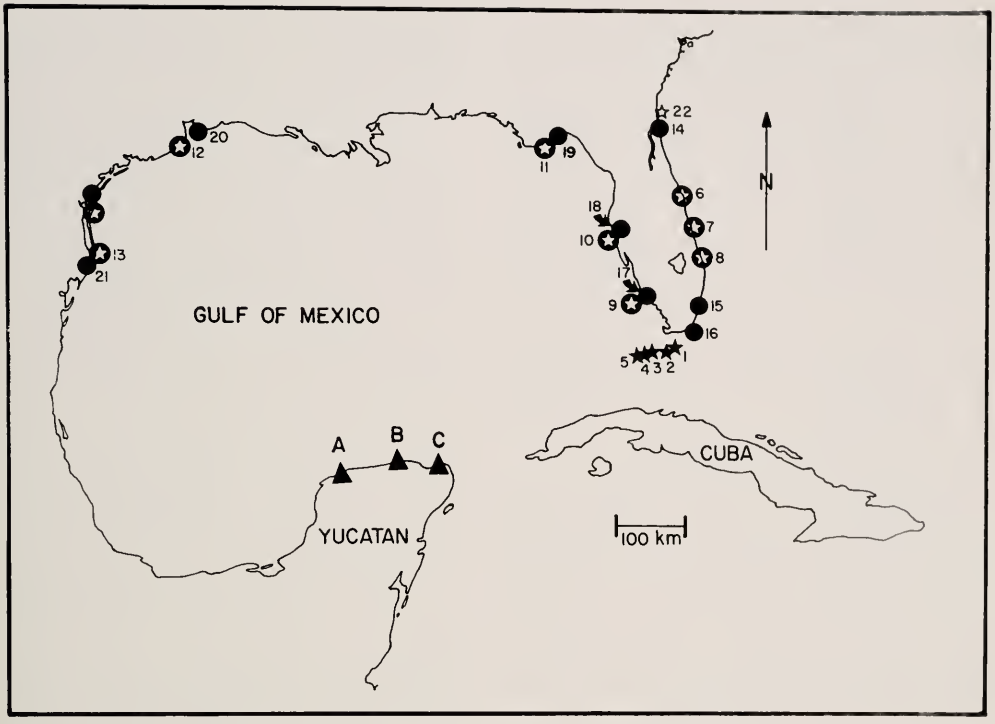

Fig. 1. Collection localities. Numbers and letters refer to populations used for electrophoresis (see Methods and Materials). Symbols: ● = *M. beryllina*, ▲ = *M. colei*, ★ = *M. conchorum*, ✪ = *M. peninsulae*, ☆ = *M. menidia*.

tion was subdivided into two groups of at least 20 individuals per group. One sub-group was preserved in 10% buffered formalin and used for morphological analysis. The other subgroup was placed in a plastic bag, covered with the water in which it was collected, and placed on a block of dry ice until returned to the laboratory. Once in the laboratory, the preserved specimens were washed overnight with tap water and transferred to 40% isopropanol, and the frozen specimens were stored at –70° C until used for electrophoresis.

LOCALITIES.—*Menidia conchorum:* Florida, Monroe County: population number 1) Long Key (20 specimens for electrophoresis; 40 specimens for morphological analysis); 2) Grassy Key (20;24); 3) Big Pine Key (20;30), 4) Cudjoe Key (20;26), and 5) Rockland Key (20;29).

Menidia peninsulae: Florida: 6) Brevard Co., Indian River near Titusville (20;30); 7) Brevard Co., Sebastian Inlet (20;35); 8) St. Lucie Co., Indian River at Ft. Pierce (20;41); 9) Collier Co., Tigertail Beach, Marco Island (20;30); 10) Hillsborough Co., Anna Maria Beach, Tampa Bay (20;30); and 11) Wakulla Co., St. Marks Lighthouse (20;30). Texas: 12) Galveston Co., San Luis Pass (20;30), 13) Cameron Co., Brownsville (20;30), and one additional population not used for electrophoresis from Aransas Co., Corpus Christi beach at Rockport (30).

Menidia beryllina: Florida: 14) Duval Co., Jacksonville (20;30), 15) Broward Co., Deerfield Beach (20;24); 16) Monroe Co., Key Largo (20;35); 17) Collier

Co., 2 km north of Marco Island (20;30); 18) Hillsborough Co., Alafia River at Federal Highway 41, Tampa Bay (20;26); and 19) Wakulla Co., St. Marks Lighthouse (20;30). Texas: 20) Chambers Co., Gilchrist Inlet (20;30); 21) Galveston Co., San Luis Pass (20;30); and two localities not used for electrophoresis, one from Florida, Dade Co., Miami, pond at University of Miami (29) and one from Texas, Nueces Co., Corpus Christi, Mustang Island State Park (30).

Menidia menidia: 22) Florida, Duval Co., St. Johns River (20;30).

Menidia colei: Mexico: A) 2.1 km south of Progreso (20;35); B) Rio Lagartos, beach on west side of city (20;35); and C) State of Quintana Roo, Chiquila (20;40).

Morphological Methods.— Counts and measurements, taken from the left side of the body, followed Hubbs and Lagler (1958). Measurements from adult specimens (greater than 35mm SL) were recorded to the nearest 0.1mm with dial calipers and were converted to a percentage of standard length. Measurements are not reported as none, in our opinion, provided significant data, but are available in Duggins, 1980 (unpublished Ph.D. dissertation, Florida State University) or from the senior author upon request. Four meristic characters were of interest in this study: number of anal fin rays, predorsal scales, lateral scales, and vertebrae (recorded from radiographs). Other traits did not yield significant results and are not reported. These data are also available from the senior author upon request.

Meristic data were analyzed by comparision of mean values by a t-test with significant level set at $p<0.01$.

Specimens used for morphological analysis were deposited in the Ichthyological collection at Florida State University, now housed at the Florida State Museum, Gainesville.

Electrophoretic Methods.— To obtain protein samples for electrophoresis, entire individuals 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 hours prior to electrophoretic separations. At the conclusion of the study, individual tissues (liver, muscle, eye) were subjected to electrophoretic separations to ascertain the source of each isozyme (Table 1).

Table 1 lists the 27 loci coding for proteins surveyed in this study. 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 Adh, GA-3-pdh, Sod, Ak, G-3-pdh, Gldh, Icdh, Mdh, Pgdh, Pgm and Xdh were surveyed on the tris-citrate-EDTA pH 7.1 ion system described by Ayala et al. (1972). Staining for Gldh was modified from Buth and Berg (1979) to contain 9.0g D-glucose, 0.005g each of NBT, MTT, PMS and 0.01g of NADP. 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. Average

TABLE 1. Enzymes systems surveyed in this study.

Enzyme	Locus	Tissue examined
Alcohol dehydrogenase	Adh	liver
Adenylate kinase	Ak	muscle
Esterase	Est-1,2	liver/muscle
Glucose dehydrogenase	Gldh	liver
Glucosephosphate isomerase	Gpi-A	muscle
	Gpi-B	muscle
Glyceraldehyde-3-phosphate dehydrogenase	GA-3-pdh-A	muscle
	GA-3-pdh-C	liver
alpha-Glycerophosphate dehydrogenase	G-3-pdh-B	liver
Isocitrate dehydrogenase	M-Icdh-A	muscle
	S-Icdh-A	liver
Lactate dehydrogenase	Ldh-A	muscle
	Ldh-B	muscle
	Ldh-C	eye
Malate dehydrogenase (NAD-dependent)	S-Mdh-A,B	eye/liver/muscle
Non-enzymatic proteins	Gp-1,2,3,4	liver/muscle
Superoxide dismutase	Sod	liver
Phosphoglucomutase	Pgm-A,B	muscle
Phosphogluconate dehydrogenase	Pgdh-A	muscle
Xanthine dehydrogenase	Xdh-A	liver
	Xdh-B	muscle

genetic distances ($\bar{D}$) and standard deviations were derived from the standard genetic distance matrix as the arithmetic means and the standard deviations about those means.

Status

Menidia conchorum is an elusive fish, thus collections of this fish have been few. The species is, however, common and found in protected mangrove bordered coves of the lower Florida Keys, notably Rockland, Cudjoe and Big Pine Keys. We feel that further sampling would show that it occurs throughout the lower Florida Keys. In our fieldwork we also found large populations in the middle Florida Keys (Grassy and Long Keys), in ecological conditions similar to those of the lower Florida Keys. The species forms fast moving schools during the day. This plus the soft coralline marl substrate make them difficult to seine. The Key Silverside is much more easily caught by dipnet at night from a shallow draft boat, a method that was successful for us.

It is probable that much habitat suitable for *M. conchorum* has been destroyed by road construction and commercial development, especially in the upper Florida Keys and around Key West. Of note is that Fowler (1945) reported *Menidia* (possibly *M. conchorum*) from Big (Upper) Matecumbe Key, but Robbins (1969) was unable to confirm that record. Fowler (1945) clearly delineated between *Menidia* and *Atherinomorus* (the Hardhead Silverside) in his monograph, and we have no reason to doubt the validity of the Big Matecumbe record of *Menidia*. This Key is only a few kilometers up the Florida Keys from Long Key, the current northernmost Key known to have a population of *M. conchorum*. The species may still exist at upper Matecumbe Key, or it could have been extirpated. It is also possible that Fowler's (1945) record could have been *M. beryllina*, which we have collected still farther north (Key

Largo), but not as far southward in the Florida Keys as Big Matecumbe.

It is unlikely that *M. conchorum* is restricted to the Florida Keys contacted by Federal Highway 1; there are probably populations on outlying Florida Keys. Although individual Key Silverside populations are seemingly large, we would caution that these populations should still be considered endangered, even though we believe that Florida Keys populations represent southernmost *M. peninsulae* (see conclusions).

Analysis of Meristic Variation

Four meristic traits are reported in the present paper (Tables 2-5). Within the Tables, populations of each species are grouped, and within a species the populations are reported from north to south on the Florida east coast and in the Florida Keys; thence around the Gulf of Mexico from east to west and into Yucatan (see Figure 1). The Tables indicate statistically significant ($p<0.01$) differences between mean values of geographically adjacent populations. Important statistically significant mean values not indicated in the Tables are: anal fin rays—1) Long Key *M. conchorum* vs. Marco Island *M. peninsulae* and Marco Island *M. beryllina;* Long Key *M. conchorum* vs. Key Largo *M. beryllina* 2) Marco Island *M. peninsulae* vs. Ft. Pierce *M. peninsulae;* Marco Island *M. peninsulae* vs. Marco Island *M. beryllina* 3) Marco Island *M. beryllina* vs. Key Largo *M. beryllina* 4) *M. colei* vs. Florida Keys *M. conchorum* and Brownsville populations of *M. beryllina* and *M. peninsulae.* Predorsal scales—1) Long Key *M. conchorum* vs. Marco Island *M. beryllina* 2) Marco Island *M. beryllina* vs. both Key Largo *M. beryllina* and Marco Island *M. peninsulae* 3) *M. colei* vs. Florida Keys *M. conchorum* and Brownsville populations of both *M. beryllina* and *M. peninsulae.* Lateral scales—1) Long Key *M. conchorum* vs. Marco Island *M. peninsulae,* Marco Island *M. beryllina* and Key Largo *M. beryllina* 2) Marco Island *M. peninsulae* vs. both Ft. Pierce *M. peninsulae* and Marco Island *M. beryllina* 3) Marco Island *M. beryllina* vs. Key Largo *M. beryllina* 4) *M. colei* vs. Florida Keys *M. conchorum* and Brownsville populations of both *M. peninsulae* and *M. beryllina.* Vertebral number 1) Long Key *M. conchorum* vs. Marco Island populations of both *M. beryllina* and *M. peninsulae* 2) Marco Island *M. beryllina* vs. Marco Island *M. peninsulae* and Key Largo *M. beryllina* 3) *M. colei* vs. Florida Keys *M. conchorum* and Brownsville populations of *M. beryllina* and *M. peninsulae.*

Meristic traits in *Menidia* are exceedingly difficult to analyze. Within a given species, some populations can be more different from other populations than they are from nearby or sympatric populations of a different species. For example, for the four meristic traits presented here (Tables 2-5), Key Largo *M. beryllina* are quite different from all other *M. beryllina.* However, they also differ from *M. peninsulae,* and are electrophoretically clearly *M. beryllina.* They are, however, more similar to adjacent *M. conchorum* in meristic traits, but not electrophoretically. Similarly, the Rio Lagartos population of *M. colei* is markedly different for the four meristic features from other *M. colei* populations, but does not differ electrophoretically. In *Menidia,* there is little intraspecific electrophoretic variation, but there is marked intraspecific meristic variation, either clinally or within a local population for no evident reason.

Analysis is presented here as variation within a species and then by a species to species comparision, followed by a summary.

TABLE 2. Number of anal fin rays in *Menidia* populations. An asterisk or an x next to consecutive populations indicates statistically significant ($p<0.01$) difference between mean values. Other such differences are indicated in the text.

Population	9	10	11	12	13	14	15	16	17	18	19	20	N	$\bar{X}$	s
beryllina-Atlantic (Florida)															
Jacksonville							5	12	12	1			30	16.3	0.8
Deerfield							3	10	7	3	1		24	16.5	1.0
Miami								7	10	8	4		29	17.3	1.0*
Key Largo						8	18	9					35	15.0	0.7*
peninsulae-Atlantic (Florida)															
Titusville						3	15	11	1				30	15.3	0.7
Sebastian Inlet						3	10	9	12	1			35	15.9	1.1
Fort Pierce						8	11	16	6				41	15.5	1.0*
conchorum-Florida Keys															
Long Key					9	24	6	1					40	13.9	0.7*
Grassy Key					11	11	2						24	13.6	0.7x
Big Pine Key				8	13	8	1						30	13.1	0.8x
Cudjoe Key				5	14	7							26	13.1	0.7
Rockland Key				8	15	5	1						29	12.9	0.8
TOTAL				21	62	55	10	1					148	13.4	0.7
peninsulae-Gulf of Mexico															
Marco Island (Florida)					2	12	12	6					30	14.7	0.9
Tampa (Florida)						10	7	10	3				30	15.2	1.0
St. Marks (Florida)						2	9	12	7				30	15.8	0.9*
San Luis Pass (Texas)							5	2	10	10	1	1	29	17.2	1.4*
Rockport (Texas)							3	9	10	5	3		30	16.9	1.1
Brownsville (Texas)							4	13	10	2	1		30	16.4	0.9
beryllina-Gulf of Mexico															
Marco Island (Florida)						1	6	14	7	2			30	16.1	0.9
Tampa (Florida)						1	2	12	10	1			26	16.3	0.8
St. Marks (Florida)						1	7	14	5	2	1		30	16.1	1.1*
Gilchrist (Texas)								12	11	6	1		30	16.9	0.9*
Corpus Christi (Texas)								14	13	11	3		30	16.6	0.7
Brownsville (Texas)							1	6	14	6	2	1	30	17.2	1.1
colei-Yucatan															
Progreso	1	12	22	5									40	10.8	0.7*
Rio Lagartos			8	20	12								40	12.1	0.7*x
Chiquila	3	14	19	2									38	10.5	0.7x
TOTAL	4	26	49	27	12								118	11.1	1.0

TABLE 3. Number of predorsal scales in *Menidia* populations. An asterisk or an x next to consecutive populations indicates statistically significant ($p<0.01$) difference between mean values. Other such differences are indicated in the text.

Population	9	10	11	12	13	14	15	16	17	18	19	N	$\bar{X}$	s
beryllina-Atlantic (Florida)														
Jacksonville									22	7	1	30	17.3	0.5*
Deerfield						1	7	14	3			25	15.7	0.7*
Miami							8	13	4			25	15.8	0.7x
Key Largo					2	10	16	6				34	14.8	0.8x
peninsulae-Atlantic (Florida)														
Titusville						11	11	8				35	14.9	0.8
Sebastian Inlet						8	24	4				35	14.9	0.6
Fort Pierce					2	16	18	3	1			40	14.6	0.8
conchorum-Florida Keys														
Long Key					3	18	12	5				38	14.5	0.8*
Grassy Key					7	13	5					25	13.9	0.7*x
Big Pine Key				1	19	10						30	13.3	0.5x
Rockland Key				8	12	7	2					29	13.1	0.9
TOTAL				9	41	48	19	5				122	14.0	0.9
peninsulae-Gulf of Mexico														
Marco Island (Florida)						10	11	5				26	14.8	0.7
Tampa (Florida)						7	16	3				26	14.9	0.6
St. Marks (Florida)						3	19	7	1			30	15.2	0.7*
San Luis Pass (Texas)						1	7	17	4	1		30	15.9	0.8*
Rockport (Texas)						1	8	16	5			30	15.8	0.8
Brownsville (Texas)						1	8	13	7			29	15.9	0.8
beryllina-Gulf of Mexico														
Marco Island (Florida)							5	14	8	2		29	16.2	0.8*
Tampa (Florida)					1	1	4	17	4			27	15.8	0.9*x
St. Marks (Florida)							4	12	13	1		30	16.4	0.8x
Gilchrist (Texas)							4	13	11	2		30	16.4	0.8*
Corpus Christi (Texas)							15	10	5			30	15.7	0.8*
Brownsville (Texas)						1	16	7	4	1	1	30	15.7	1.1
colei-Yucatan														
Progreso	2	26	8	3								39	10.3	0.7*
Rio Lagartos		10	17	10	2							39	11.1	0.9*
Chiquila	1	14	18	5								38	10.7	0.7
TOTAL	3	50	43	18	2							116	10.7	0.7

TABLE 4. Number of lateral scales in *Menidia* populations. An asterisk or an x or y next to consecutive populations indicates statistically significant ($p<0.01$) difference between mean values. Other such differences are indicated in the text.

Population	28	29	30	31	32	33	34	35	36	37	38	39	40	41	N	$\bar{X}$	s
beryllina-Atlantic (Florida)																	
Jacksonville										4	8	12	5	1	30	38.7	1.0*
Deerfield								5	8	8	4				25	36.4	1.0*x
Miami									2	12	16	1			31	37.3	0.7xy
Key Largo						2	13	15	3						33	34.6	0.8y
peninsulae-Atlantic (Florida)																	
Titusville									4	10	11	4			29	37.5	0.9
Sebastian Inlet									1	12	13	8	1		35	37.9	0.9*
Fort Pierce									5	28	5	3			41	37.2	0.7*x
conchorum-Florida Keys																	
Long Key							4	18	12	3					37	35.4	0.8x
Grassy Key							5	15	4	1					25	35.0	0.7
Big Pine Key						2	10	14	3						29	34.6	0.8*
Cudjoe Key						10	10	5							25	33.8	0.8*
Rockland Key						5	17	4	1						27	34.0	0.7
TOTAL						17	46	56	20	4					143	34.6	0.9
peninsulae-Gulf of Mexico																	
Marco Island (Florida)									1	7	18	3			29	37.8	0.7*
Tampa (Florida)										1	12	9	3		28	38.4	0.9*
St. Marks (Florida)											9	15	3	1	28	38.9	0.8x
San Luis Pass (Texas)										6	14	7	2		29	38.2	0.9x
Rockport (Texas)										6	15	8			29	38.1	0.7*
Brownsville (Texas)									3	10	15	2			30	37.5	0.8*
beryllina-Gulf of Mexico																	
Marco Island (Florida)									9	18	2	1			30	36.8	0.7*
Tampa (Florida)										3	18	3	1		25	38.1	0.7*x
St. Marks (Florida)										13	12	3			28	37.6	0.7xy
Gilchrist (Texas)								1	8	12	6				27	36.9	0.8y
Corpus Christi (Texas)									2	23	4				29	37.1	0.5
Brownsville (Texas)									4	15	9	1			29	37.2	0.7
colei-Yucatan																	
Progreso	1		13	22	2										38	30.6	0.7*
Rio Lagartos					9	14	10	2							35	33.1	0.9*x
Chiquila		1	16	16	7										40	30.7	0.8x
TOTAL	1	1	29	38	18	14	10	2							113	31.4	1.9

TABLE 5. Number of vertebrae in *Menidia* populations. An asterisk or an x, y or z next to consecutive populations indicates statistically significant ($p<0.01$) difference between mean values. Other such differences are indicated in the text.

Population	32	33	34	35	36	37	38	39	40	41	42	N	$\bar{X}$	s
beryllina-Atlantic (Florida)														
Jacksonville							1	8	9	12		30	40.1	0.9*
Deerfield						3	7	9	3			22	38.6	0.9*
Key Largo					2	16	13	2				33	37.5	0.7
peninsulae-Atlantic (Florida)														
Titusville							6	12	9	3		30	39.3	0.9
Sebastian Inlet							4	15	12	3	1	35	39.5	0.9
Fort Pierce							4	24	1	1		30	39.0	0.6*
conchorum-Florida Keys														
Long Key				1	4	8	16	1				30	37.4	0.9*
Grassy Key					2	15	7	1				25	37.3	0.7x
Big Pine Key			1	2	13	9	2					27	36.3	0.9x
Rockland Key					17	11	1					29	36.5	0.6
TOTAL			1	3	36	43	26	2				111	36.9	0.8
peninsulae-Gulf of Mexico														
Marco Island (Florida)							2	16	5	4		27	39.4	0.8
Tampa (Florida)							1	9	11	7	1	29	39.9	0.9*
St. Marks (Florida)									14	13	3	30	40.6	0.7*x
San Luis Pass (Texas)								13	13	3	1	30	39.2	0.8xy
Rockport (Texas)						1		3	18	8		30	40.1	0.8yz
Brownsville (Texas)							3	14	12	1		30	39.4	0.7z
beryllina-Gulf of Mexico														
Marco Island (Florida)						2	20	5	2			29	38.2	0.7*
Tampa (Florida)								13	12	1		26	39.5	0.6*
St. Marks (Florida)							5	9	15	1		30	39.4	0.8x
Gilchrist (Texas)						4	10	13	3			30	38.5	0.9x
Corpus Christi (Texas)						3	3	22	2			30	38.8	0.7
Brownsville (Texas)					1	4	9	12	3			29	38.4	1.0
colei-Yucatan														
Progreso	4	15	9	2								30	33.3	0.8*
Rio Lagartos			1	16	11		1					29	35.5	0.7*x
Chiquila	1	11	15	3								30	33.7	0.7x
TOTAL	5	26	25	21	11		1					89	34.1	1.4

INTRASPECIFIC VARIATION.—*Menidia beryllina*—Considerable inter-population variation exists with statistically different means between adjacent populations, i.e. Miami and Key Largo, and Key Largo and Marco Island, for anal fin rays; between Jacksonville and Deerfield, Miami and Key Largo, Key Largo and Marco Island, and Marco Island and Tampa for predorsal scale number; between Miami and Key Largo, Key Largo and Marco Island, and Marco Island and Tampa for lateral scale number; and between all adjacent Atlantic coast populations, and Key Largo and Marco Island, and Marco Island and Tampa for vertebral number. In general, there are sharp clinal patterns with decreasing counts southward on the Atlantic coast, but there is less marked variation in the Gulf of Mexico. There is not much similarity between Gulf of Mexico and Atlantic coast populations, and the southernmost population at Key Largo has markedly lower, statistically significant, counts for all traits.

Menidia peninsulae—Between population variation is less marked in this species than in *M. beryllina*. Atlantic coast populations are rather uniform for the traits studied. Statistically significant differences exist for all traits between eastern and western Gulf of Mexico populations, a fact also noted by Chernoff et al. (1981). Some differentiation between Gulf of Mexico and Atlantic populations is also indicated. North-south clinal patterns are not evident, in contrast to *M. beryllina*.

Menidia conchorum—Middle Florida Keys populations tend to have higher counts than lower Florida Keys populations. Some clinal gradation is evident, especially for lateral scale number. Middle and lower Florida Keys populations do differ at a significant level for predorsal scales and vertebral number. The data do not suggest, however, any more marked inter-population variation than occurs in *M. peninsulae*, and less than in *M. beryllina*.

Menidia colei—In all traits examined the Rio Lagartos population had markedly higher means than the Progreso and Chiquila populations. Based on these four traits alone, the Rio Lagartos population could certainly be recognized as specifically distinct from the other two. This is at odds with the electrophoretic data presented later in this paper, however, and no conclusion is drawn from the meristic data alone at this point. It should be emphasized that the meristic differences exhibited by the Rio Lagartos population (as compared to other *M. colei* populations) is no greater than that shown by the Key Largo population of *M. beryllina* (as compared to other *M. beryllina* populations).

The meristic variation exhibited by the above *Menidia* species is remarkable. It may reflect a considerable influence of environmental conditions on meristic traits during development, genetic drift or some especially strong local selection. Our data are in strong support of Chernoff et al.'s (1981) conclusion that *Menidia* are phenotypically plastic.

INTERSPECIFIC COMPARISIONS.—*Menidia beryllina* and *M. peninsulae*—Although a comparision of these species has been done by others (Johnson, 1975), we include brief observations which have a bearing on the relationship of *M. conchorum* to both species. Atlantic coast populations of *M. beryllina* and *M. peninsulae* taken as a whole have statistically different means for anal fin ray number, predorsal scale number and number of vertebrae. Key Largo populations of *M. beryllina* are more similar for anal fin rays and predorsal

scale number to *M. peninsulae* than to other *M. beryllina* populations. However, these Key Largo *M. beryllina* differ markedly from *M. peninsulae* in number of lateral scales and vertebrae. Gulf of Mexico populations of *M. beryllina* and *M. peninsulae* differ in number of predorsal and lateral scales, but meristic differences in general are not as great as between Atlantic coast populations of the two species.

Menidia beryllina and *M. conchorum*—Comparision of *M. beryllina* populations adjacent to *M. conchorum* involves comparision of Key Largo and Marco Island *M. beryllina* to Long Key *M. conchorum*. Key Largo *M. beryllina* differ significantly from Long Key populations in the number of anal fin rays and lateral scales, while Marco Island *M. beryllina* differ significantly from Long Key *M. conchorum* in all four traits presented here. For one trait, number of predorsal scales, Key Largo *M. beryllina* and Long Key *M. conchorum* are quite similar, but so is Ft. Pierce *M. peninsulae*. Of note is that *M. beryllina* from Key Largo, Ft. Pierce *M. peninsulae* and Long Key *M. conchorum* are all more similar to one another for the number of predorsal scales than middle Florida Keys *M. conchorum* are to lower Florida Keys *M. conchorum*. For number of vertebrae, Key Largo *M. beryllina* are also quite similar to *M. conchorum*, while *M. peninsulae* differs significantly from *M. conchorum* for this trait.

In summary, Key Largo *M. beryllina* are more similar in meristic traits to *M. conchorum* from Long Key than to *M. beryllina* from Marco Island, and middle Florida Keys *M. conchorum* are more similar to Key Largo *M. beryllina* than to lower Florida Keys *M. conchorum*. We feel that these counts are quite probably under environmental influence—different species (i.e. *M. beryllina* and *M. conchorum*) from the same or nearby localities are more similar than populations of the same species from different localities.

Menidia peninsulae and *M. conchorum*—The Fort Pierce (Atlantic coast) population of *M. peninsulae* is statistically different in the number of anal fin rays, lateral scales and vertebrae from Long Key *M. conchorum*. The same three traits distinguish Marco Island *M. peninsulae* from Long Key *M. conchorum*. The magnitude of these differences could be interpreted as that of species level differentiation, although clinal variation, with counts decreasing southward, is evident on the Florida Gulf coast. Electrophoretic data presented later, however, show no differences between *M. peninsulae* and *M. conchorum*.

Menidia colei and *M. conchorum, M. beryllina* and *M. peninsulae*—*Menidia colei* can be clearly distinguished from these three other species on the basis of all four traits presented. There is no basis, from meristic data, to conclude a closer relationship of *M. colei* to *M. conchorum* or to either *M. beryllina* or *M. peninsulae* populations from southern Texas.

In summary, meristic data clearly delineate *M. conchorum* from *M. colei* and to a lesser degree from *M. beryllina* and *M. peninsulae*. *Menidia conchorum* is as different from *M. peninsulae* in meristic traits as it is from southernmost *M. beryllina* populations. In fact, middle Florida Keys *M. conchorum* are more similar overall to Key Largo *M. beryllina* then to either *M. peninsulae* or lower Florida Keys *M. conchorum*, indicating an environmental influence on meristics. This statement ignores, however, the north-south clinal trends in *M. conchorum* and *M. peninsulae* and the allopatry of *M. conchorum* and *M. beryllina*.

The only decision relative to *M. conchorum* that can be made with these

TABLE 6. Electromorph frequencies in *Menida* (Population symbols: co=*M. colei*, cm=*M. conchorum*, p=*M. peninsulae*, b=*M. beryllina*, m=*M. menidia*).

		co	1cm	2cm	3cm	4cm	5cm	6p	7p	8p	9p	10p	11p	12p
Gpi-A	*a*				0.03			0.12	0.03		0.12			
	b	0.04		0.70	0.10	0.70	0.05	0.65	0.17	0.17	0.58	0.23	0.07	0.15
	c	0.96	0.55	0.20	0.80	0.27	0.90	0.20	0.50	0.53	0.25	0.50	0.50	0.77
	d		0.45	0.10	0.07	0.03	0.05	0.03	0.30	0.30	0.05	0.23	0.38	0.08
	e											0.04	0.05	
Gpi-B	*a*											0.03	0.03	
	b					0.08					0.05	0.03	0.03	0.05
	c	1.0	0.80	1.0	1.0	0.84	0.68	1.0	1.0	1.0	0.95	0.94	0.94	0.95
	d		0.20			0.08	0.32							
AK	*a*													
	b	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
Est-3	*a*													
	b	1.0	0.13		0.15	0.15	0.13				0.05	0.05		
	c		0.87	1.0	0.85	0.80	0.63	1.0	1.0	1.0	0.75	0.95	1.0	1.0
	d					0.05	0.24				0.20			
	e													
Gp-1	*a*	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													
Gp-2	*a*		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	b	1.0												1.0
Gp-3	*a*													
	b	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
M-Icdh-A	*a*		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	1.0												
S-Icdh-A	*a*													
	b		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	c	1.0												
Pgm-B	*a*													
	b													
	c													
	d			0.05		0.20						0.10		
	e				0.03				0.10		0.03		0.03	
	f	0.98	1.0	0.93	0.97	0.70	1.0	1.0	0.85	1.0	0.97	0.85	0.97	0.05
	g	0.02		0.02		0.10			0.05			0.05		0.95

meristic data is that *M. conchorum* is a distinct allopatric species, a decision that is at odds with the electrophoretic data that follow. The overall high level of intraspecific variation with *Menidia* meristic data renders this conclusion suspect, in our opinion, and must be tempered by analysis of the electrophoretic data.

Electrophoretic Variation.—Of the 27 loci coding for proteins surveyed in this study, 5 (Gp-4, Adh, GA-3-pdh, Sod and Ldh-C) were fixed for the same electromorph in all samples. For an additional four loci (Ldh-A,B, S-Mdh-A and Pgdh-A) the same electromorph was found in at least 98% frequency in all samples; these four loci are also con-

TABLE 6. (Continued)

13p	14b	15b	16b	17b	18b	19b	20b	21b	m
		0.25	0.52	0.03		0.02			
0.17	0.05	0.03		0.93	0.58	0.42	0.15		0.15
0.63	0.48	0.48	0.48	0.04	0.40	0.50	0.80	1.0	0.05
0.20	0.45	0.24			0.02	0.06	0.05		0.75
	0.02								0.05
	0.03								0.93
0.05	0.20						0.03		0.07
0.93	0.77	0.90	0.98	0.95	1.0	1.0	0.97	1.0	
0.02		0.10	0.02	0.05					
	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	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
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	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
									1.0
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
									0.5
									0.5
	0.05				0.20				
	0.95	1.0	1.0	1.0	0.80	1.0	0.90	1.0	
							0.10		
1.0	0.05								

sidered monomorphic. Electromorph frequency variation was observed for the remaining 18 loci studied. In six of the polymorphic loci (G-3-pdh-B, S-Mdh-B, Pgm-A, Xdh-A,B and GA-3-pdh-C) there was only slight electrophoretically detectable variation. The common shared electromorph frequency varied from fixation to 63%. These above loci are not presented in tabular form. For two loci (Gldh and Est-1) no single electromorph was the most common in all samples. The number of observed electromorphs per locus varied from four (Gldh) to six (Est-1). Unique, rare and semi-rare, frequencies less than 25%, electromorphs were found only in populations of *M. beryllina*. Although populations of *M. conchorum* were variable for Gldh, they

possessed no electromorphs that were not also found in either *M. peninsulae* or *M. beryllina*. Data for these loci are also not presented in tabular form.

Electromorph frequencies for Gpi-A,B demonstrated marked perturbations among samples. A rare electromorph (*a*) for Gpi-B was found at a nearly fixed difference level in *M. menidia* (93%). *Menidia conchorum* and *M. peninsulae* were similar for Gpi-A,B. (Table 6).

Alternative species specific electromorphs were found for Ak, Gp-1,2,3, M-Icdh-A, S-Icdh-A, Pgm-B and Est-3 (Table 6). *Menidia beryllina* was completely differentiated from all other samples by electromorphs for Est-3 and from all except *M. menidia* by Ak. In addition, Pgm-B affords a nearly fixed difference from *M. peninsulae* (and *M. conchorum*). As noted by Johnson (1975), Texas populations of *M. peninsulae* share the otherwise species specific Gp-2 (*b*) electromorph found in *M. beryllina*. Although the above three (or four including Gp-2) loci clearly differentiate *M. beryllina* and *M. peninsulae*, no species specific electromorphs were found to differentiate *M. peninsulae* and *M. conchorum*. There is no evidence from the genetic data to suggest *M. conchorum* hybridizes with *M. beryllina;* no heterozygotes were observed for Ak, Gp-2 or Est-3. No evidence was found to suggest that *M. conchorum* and *M. peninsulae* were differentiated to the degree that hybridization could be detected by electrophoretic analysis.

Menidia menidia is completely differentiated by Est-3, Gp-1,3, S-Icdh-A and Pgm-B from other *Menidia*. *Menidia colei* is differentiated from all other populations by M-Icdh-A, S-Icdh-A and shows marked frequency differences from other *Menidia* species at Est-3. Three populations of *M. colei* were analyzed and showed virtually no interpopulation variation (D=0.008). In summary, *M. conchorum* differs from *M. beryllina* and *M. colei* at four loci, and from *M. menidia* at eight. However, *M. conchorum* and *M. peninsulae* do not have any fixed differences and show no indication of major gene frequency differences.

Because closely related species that have begun to diverge may be differentiated by only subtle differences in gene frequencies (Ayala et al., 1974; Avise, 1974; Mayr, 1963), genetic distance estimates, D, were calculated from the electromorph frequency data. Values for pairwise comparisions within and between species are reported in Table 7. Interspecific values ranged from 0.027 (*M. conchorum* vs. *M. peninsulae*) to a maximum of 0.394 (*M. colei* vs. *M. menidia*). The genetic distance of *M. colei* to *M. peninsulae*, *M. conchorum*, and *M. beryllina* was 0.177, 0.205, and 0.241, respectively. *Menidia colei* is not clearly more closely related to one than the other, and certainly there is no evidence from biochemical data that *M. colei* and *M. conchorum* are especially closely related and should be separated into another genus.

TABLE 7. Mean genetic distance within and between 5 species of *Menidia*.

	colei	*conchorum*	*beryllina*	*peninsulae*	*menidia*
colei	0.005±0.004	0.205±0.007	0.241±0.031	0.177±0.005	0.394±0.013
conchorum		0.024±0.011	0.195±0.024	0.027±0.022	0.383±0.02
beryllina			0.028±0.013	0.200±0.102	0.285±0.04
peninsulae				0.027±0.021	0.373±0.05
menidia					—

The average standard genetic distances ($\bar{D}$) within taxa were small and consistent: $\bar{D}$=0.024± 0.011 for *M. conchorum;* 0.027± 0.021 for *M. peninsulae;* 0.028± 0.013 for *M. beryllina,* and 0.005± 0.004 for *M. colei.* The $\bar{D}$ values between either *M. conchorum* or *M. peninsulae* and *M. beryllina* ($\bar{D}$=0.195± 0.024 and 0.200± 0.013 for *M. conchorum* and *M. peninsulae* vs. *M. beryllina,* respectively), were similar and an order of magnitude greater than within taxa values. However, $\bar{D}$ values between *M. conchorum* and *M. peninsulae* ($\bar{D}$=0.027± 0.022) were an order of magnitude smaller than the values observed between other taxa. This smaller value is on the order of values observed between conspecific fish populations (Avise, 1974).

We interpret these results to corroborate the initial genetic result, that of no genetic differentiation between *M. conchorum* and *M. peninsulae.* We and others (Johnson, 1975) have demonstrated that *M. peninsulae* and *M. beryllina* are readily distinguishable species based on biochemical characters and we observe large average genetic distances between them (D=0.200). On the other hand, no species specific electromorphs were found to differentiate *M. conchorum* from *M. peninsulae,* and both of these forms are differentiated from *M. beryllina* by the same set of traits. The smallest genetic distance values were observed between *M. conchorum* and *M. peninsulae.* Furthermore, the observed genetic distance values between these nominal species were on the order expected and observed between conspecific populations.

Systematic Conclusions

Johnson (1975) studied morphological and electrophoretic characters in five species of *Menidia.* He concluded that *M. peninsulae* and *M. beryllina* were sibling species, but he did not consider the systematic position of *M. conchorum.* He followed Miller (1976) and accepted the view that Key Silversides were rare and restricted to the lower Florida Keys. As noted earlier, our field collections revealed populations of *M. conchorum* in the middle Florida Keys (Long and Grassy Keys), thus suggesting a more wide-spread and northerly distribution than previously recognized.

The possibility that the populations of *M. conchorum* we found in the middle Florida Keys represent recently founded or introduced populations should be considered. However, we know of no basis for such an assumption. In such cases of founder populations, a genetic "bottleneck" may be created and heterozygosity is depressed as compared to long established populations (Soule, 1976). We calculated average individual heterozygosity H (Soule and Yang, 1973) for *M. conchorum* and the populations of the other species of *Menidia* examined in this paper. Average heterozygosities (Table 8) in all species populations ranged from 1.13% (Brownsville, Texas population of *M. beryllina*) to 10.20% (Tampa, Florida population of *M. peninsulae*); the heterozygosities for Grassy Key (2.88%) and Long Key (4.11%) were not depressed. Based on the robustness of the middle Florida Keys populations as observed in the field and the lack of depressed heterozygosities, we conclude that there is no evidence that middle Florida Keys populations of *M. conchorum* represent newly founded populations. We suggest collecting efforts in the past were inadequate, and that other, as yet undiscovered northern populations and populations on outlying Florida Keys, exist.

TABLE 8. Heterozygosity values for populations of four species of *Menidia*.

Locality	Population No.	H
colei		
Progreso	A	1.88
Rio Lagartos	B	1.20
Chiquila	C	3.60
peninsulae (formerly *conchorum*)		
Long Key	1	4.11
Grassy Key	2	2.88
Big Pine Key	3	4.62
Cudjoe Key	4	5.40
Rockland Key	5	5.74
peninsulae		
Titusville	6	3.70
Sebastian Inlet	7	6.35
Ft. Pierce	8	3.33
Marco Island	9	6.32
Tampa	10	10.20
St. Marks	11	4.04
San Luis Pass	12	5.00
Brownsville	13	5.28
beryllina		
Jacksonville	14	7.58
Deerfield	15	7.22
Key Largo	16	5.56
Marco Island	17	4.60
Tampa	18	8.46
St. Marks	19	4.44
Gilchrist	20	4.11
Brownsville	21	1.13
menidia		
Jacksonville	22	6.01

We reach the following conclusions:

1. *Menidia conchorum* and *M. peninsulae* differ on the basis of meristic features to a level that could by traditional taxonomic analysis be considered distinct species. However, these nominal species show no differences in any of 27 electrophoretic traits examined, suggesting that the observed meristic variation in both *M. peninsulae* and the nominal *M. conchorum* could be due to environmental effects on the phenotype. Of note is the considerable meristic variation throughout *Menidia* that is evident in Tables 2-5 (and see Chernoff et al., 1981). In fact, based on the four meristic characters presented here, there is nearly as much justification to recognize the Rio Lagartos population of *M. colei* and the Key Largo population of *M. beryllina* as distinct species as there is to consider *M. conchorum* distinct. We believe, as did Chernoff et al. (1981) that much of the meristic variation in *Menidia* is due to environmental influences, as evidenced by north-south clinal variation and similar meristic counts in different species from the same or nearby localities. Such variation is not evident for the electrophoretic traits. This wide meristic variation, we believe, has masked the genetic similarity of *M. conchorum* and *M. peninsulae* in previous studies. In this case, the electrophoretic data are, in our opinion, more reliable and strongly suggest that *M. conchorum* is conspecific with *M. peninsulae*. We temper our conclusion, however, because we recognize that although two forms can be proven different, it is impossible to prove that they are the same, because one can never examine all possible characters. Nevertheless, we feel that *M. peninsulae* and *M. conchorum* are conspecific. The name *M. peninsulae* has nomenclatural priority.

2. *Menidia beryllina* and *Menidia peninsulae* are distinct species, as demonstrated earlier by Johnson (1975). *Menidia conchorum* differs electrophoretically from *M. beryllina* in the same ways that *M. peninsulae* does.

3. *Menidia colei* is not more closely related to *M. conchorum* than it is to other *Menidia* species, thus invalidating Schultz's (1948) suggestion that they should be grouped together in the genus *Menidiella*.

4. *Menidia conchorum* is more widespread than previously thought; the newly discovered populations do not appear to be recently founded.

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