

## Phylogenetic Assessment of *Eucinostomus gula*, *Eugerres plumieri*, and *Diapterus auratus* (Pisces: Gerreidae) Based on Allozyme and mtDNA Analyses

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**ABSTRACT.**—The phylogeny of three gerreid species was evaluated through allozymes and mtDNA analyses. Genetic similarities were estimated with data from 18 protein-coding loci and from mtDNA fragments produced by 3 restriction enzymes. The allozyme and mtDNA dendograms had identical topology. Genetic distance, genetic similarity, and mtDNA sequence divergence indicated that *E. plumieri* and *D. auratus* are closer than any other combination of the three taxa. *Eugerres* and *Diapterus* are clearly sister groups within the dendogram. Allozyme and mtDNA analyses support the common taxonomic practice of grouping the mojarras that possess a serrated preoperculum.

### INTRODUCTION

The family Gerreidae (mojarras) comprises small to medium sized, strongly compressed fishes characterized by a pointed snout with highly protrusible mouth (Nelson, 1994). Mojarras inhabit tropical and subtropical shallow coastal areas and clear waters around islands. They occur over muddy and sandy bottoms, in estuaries, hypersaline lagoons and fresh water (Cervigon et al., 1993).

The systematics of this family has a history of instability caused primarily by the unequal value given to morphological characters and the subjectivity found in some species descriptions. Most taxonomist currently recognize five genera: *Eucinostomus* Baird & Girard 1855, *Eugerres* Jordan & Evermann, 1927, *Diapterus* Ranzani 1842, *Gerres* Quoy & Gaimard 1824, and *Ulaema* Jordan & Evermann 1895, with about 13 species in the western Atlantic (Eschmeyer, 1998).

Although our understanding of Gerreid taxonomy has improved, the phylogeny within the family is poorly known. The present study examined the relationships of three gerreid genera through an indepen-

dent comparison of allozyme and mtDNA analyses. The phylogeny of *Eucinostomus gula* (Quoy & Gaimard, 1824), *Eugerres plumieri* (Cuvier, 1830), and *Diapterus auratus* (Ranzani, 1840) is evaluated.

### MATERIALS AND METHODS

Adult mojarras were captured either with a 5 m cast net or a 50 m beach seine, both 15 mm mesh. *Eucinostomus gula* and *E. plumieri* were collected in Puerto Progreso, Yucatan, Mexico (21° 10' N, 89° 30' W) and *D. auratus* in Port Charlotte, Florida. (26° 47' N, 82° 09' W). Fishes were killed on site and tissue samples were dissected and stored until analysis; samples of muscle, liver, and a whole left eye were stored at -10 C°, gonad samples were stored at -70 C°.

The analysis of allozymes involved 10 enzymatic systems (Table 1) and 31 specimens of each species. The mitochondrial DNA analysis included 10 specimens of each species. Allozyme nomenclature adheres to Shaklee et al. (1990) but asterisks are omitted from the locus abbreviation. Starch gel electrophoresis followed standard procedures (Aebersold et al., 1987;

TABLE 1. Enzymatic systems and electrophoretic settings. Allozyme names and numbers follow Shaklee et al. (1990) but asterisks are omitted.

Allozyme	Enzyme no.	Locus	Buffer <sup>1</sup> , pH	Tissue <sup>2</sup>
Aspartate aminotransferase (AAT)	2.6.1.1	AAT	TBCL, 8.0	M
Creatine kinase (CK)	2.7.3.2	CK	TBE, 8.6	M
Glycerol-3-phosphate dehydrogenase (G3PDH)	1.1.1.8	G3PDH	TBCL, 8.0	M,E
Glucose-6-phosphate dehydrogenase G6PDH	1.1.1.49	G6PDH	TBCL, 8.0	M,L
Glucose-6-phosphate isomerase (GPI-1)	5.3.1.9	GPI	TBE, 8.6	M,L
L-Iditol dehydrogenase (IDDH)	1.1.1.14	IDDH	AC, 6.9	L
L-Lactate dehydrogenase (LDH)	1.1.1.27	LDH	TBE, 8.6	M,E,L
Malate dehydrogenase (MDH)	1.1.1.37	MDH	AC, 6.9	M
Phosphoglucosmutase (PGM)	5.4.2.2	PGM	TBCL, 8.0	M
Superoxidase dismutase (SOD)	1.15.1.1	SOD	TBE, 8.6	M,E

<sup>1</sup>TBCL = Tris-HCl, TBE = Tris-borate-EDTA, AC = Amine-citrate morpholine

<sup>2</sup>E = eye; L = liver; M = muscle

Murphy et al., 1990). Enzyme staining procedures followed Murphy et al. (1990). Several measures of genetic variation were computed with Biosys-1 software (Swofford and Selander, 1981). Allozyme frequencies were examined for conformation to Hardy-Weinberg equilibrium. Allele frequencies were compared using likelihood-ratio G-statistics (Sokal and Rohlf, 1981). Unbiased genetic distances were calculated according to Nei (1978). Dendograms were constructed using UPGMA clustering method (Sneath and Sokal, 1973).

Total mtDNA was extracted from gonad tissue according to Chapman and Powers (1984). Mitochondrial DNA was stained with ethidium bromide and the separation gradients were visualized under UV light. The restriction endonucleases were: Hae III, Hind III, and Taq I. Digestion reactions were done using 20 µl of extracted mtDNA under conditions recommended by the manufacturer. The resulting mtDNA fragments were separated by electrophoresis on 1.1 % agarose gels (Maniatis et al., 1982). Phage λ DNA digested with Hind III was used as the molecular weight standard (Maniatis et al., 1982). The divergence values among sequences were calculated from the comparison among fragments (Avise et al., 1986), and grouped in clusters with UPGMA (Sneath and Sokal, 1973).

Following tissue extraction, specimens were fixed in 10 % buffered formalin and preserved in 70 % ethanol. Taxonomic

identification followed Deckert and Greenfield (1987) and Matheson and McEachran (1994). The preserved specimens were deposited in the Instituto de Biología (IB-UNAM), Universidad Nacional Autónoma de México, and the Florida Museum of Natural History, University of Florida (UF).

## RESULTS

The 10 allozyme systems revealed 32 alleles in 18 presumptive protein-coding loci (Table 2). Of the 18 protein-coding loci, 11 were monomorphic, 7 polymorphic, and 3 divergent. AAT was reactive in some samples, but showed stain deficiencies in others. There were 4 alleles and the heterozygote phenotype showed 3 bands. AAT was not included in the analysis. CK, dimeric: monomorphic in the three species. G3PDH, dimeric: monomorphic in both muscle and liver in the three species. G6PDH, dimeric: monomorphic in muscle of the three species, and polymorphic in liver with two alleles. GPI-1, dimeric: it showed 5 alleles in muscle and 3 alleles in liver. The muscle form is divergent and has diagnostic value for the three species. IDDH, tetrameric: monomorphic in the three species. LDH, tetrameric: LDH was monomorphic in muscle of the three species. LDH liver and LDH eye were polymorphic with 2 and 3 alleles respectively. LDH eye was divergent and has taxonomic

TABLE 2. Allele frequencies at 18 protein-coding loci.

Allozyme <sup>1</sup>	Allele	<i>E. gula</i> n = 31	<i>E. plumieri</i> n = 31	<i>D. auratus</i> n = 31
AAT M	*100	0.975	0	0
	125	0.025	0.045	0
	130	0	0.955	0.930
	140	0	0	0.070
CK M	*100	1.000	1.000	1.000
G3PDH M	*100	1.000	1.000	1.000
G3PDH L	*100	1.000	1.000	1.000
G6PDH-a M	*100	1.000	1.000	1.000
	*100	0.950	1.000	1.000
G6PDH-b L	125	0.050	0	0
	*100	1.000	0	0
	125	0	0	0.825
	80	0	0.043	0
	75	0	0	0.175
GPI-1 M	70	0	0.957	0
	*100	0	1.000	0.014
	130	0.862	0	0.986
	95	0.138	0	0
IDDH L	*100	1.000	1.000	1.000
LDH-a M	*100	1.000	1.000	1.000
LDH-b L	*100	1.000	0.980	1.000
	90	0	0.020	0
LDH-c E	*100	1.000	0.770	0.896
	85	0	0	0.104
	70	0	0.230	0
MDH-a M	*100	1.000	1.000	1.000
MDH-b L	*100	0.015	1.000	1.000
	115	0.985	0	0
PGM-a M	*100	1.000	1.000	1.000
PGM-b M	*100	1.000	1.000	1.000
SOD M	*100	1.000	1.000	1.000
SOD E	*100	1.000	1.000	1.000

<sup>1</sup>E = eye, L = liver, M = muscle.

value. MDH, dimeric: monomorphic in muscle, polymorphic in liver were it showed 2 alleles; it has diagnostic value. PGM-a and PGM-b, both were monomorphic in muscle of the three species. SOD, dimeric: monomorphic in both muscle and liver of the three species.

Seven allozymes: AAT muscle, G6PDH liver, GPI muscle, GPI liver, LDH liver, LDH eye, and MDH liver, were polymorphic in the gerreids studied. The genetic distances were calculated using 28 alleles and 17 protein-coding loci. The values for unbiased genetic distance and unbiased genetic identity are given in Table 3. The values for heterozygosity per locus, percentage of polymorphic loci, and Hardy-

TABLE 3. Matrix for Nei's (1978) unbiased genetic distances (above diagonal) and unbiased genetic identity (below diagonal).

Population	<i>E. gula</i>	<i>D. auratus</i>	<i>E. plumieri</i>
<i>E. gula</i>	*****	0.235	0.301
<i>D. auratus</i>	0.791	*****	0.169
<i>E. plumieri</i>	0.740	0.845	*****

Weinberg expectations are shown in Table 4. The dendrogram of genetic similarity calculated with UPGMA is shown in Figure 1.

The digestion of total mtDNA with restriction endonucleases Hae III, Hind III, and Taq I rendered 13 mtDNA fragments in *E. gula*, and 12 in *E. plumieri*, and *D. auratus*. The average mtDNA molecular

TABLE 4. Sample size and genetic variability expressed as the mean number of alleles per locus, percentage of loci polymorphic and heterozygosity of the taxa studied.

Population	n	Alleles/locus	% Polymorphic loci	Heterozygosity <sup>1</sup>	
				Observed	Expected Hardy-Weinberg
<i>E. gula</i>	31	1.4 (0.1)	16.7	0.029 (0.012)	0.034 (0.013)
<i>D. auratus</i>	31	1.2 (0.1)	11.1	0.014 (0.009)	0.023 (0.013)
<i>E. plumieri</i>	31	1.2 (0.1)	11.1	0.007 (0.004)	0.024 (0.012)

<sup>1</sup>Standard error in parentheses

weight was:  $16,970 \pm 60$  bp in *E. gula*,  $16,980 \pm 60$  bp in *E. plumieri*, and  $16,960 \pm 60$  bp in *D. auratus* (Table 5). The total number of mtDNA fragments and the number of restriction sites shared between species-pairs are given in Table 6. The dendrogram of mtDNA sequence divergence is presented in Figure 2.

#### DISCUSSION

The estimated genetic distances between these mojarras are small and fall within the range of other fish families (Avisé and Aquadro, 1982; Billington et al., 1990). We are aware of the limitations of making intergroup comparisons of genetic distance, but such comparisons have been useful for guiding taxonomic assessments (Shaklee and Tamaru, 1981). The small genetic distances between these taxa could indicate recent origin or slow rate of evolution (Avisé and Smith, 1974; Gillespie, 1987). Genetic identity and genetic distance showed that *E. plumieri* and *D. auratus* are the most genetically similar; the genetic distance between these two species under the neutral hypothesis of mutation, where the genetic distance unit for fishes corresponds to 1-18

million years (Avisé et al., 1975; Gorman et al., 1976), suggests that divergence between them occurred about 0.169-3.042 MY. The wide range of the divergence value is due to the correction factors of the protein molecular clock; these were derived from homeotherm vertebrates (Kishino and Hasegawa, 1990).

The values of average heterozygosity per locus (Table 4) were low in the gerreids studied when compared with the estimated 0.051 calculated for 183 species of fish (Nevo et al., 1984). Sarich (1977) and Gorman and Kim (1979) suggested that low heterozygosity was influenced more by the number of loci analyzed than by sample size. It is possible that low heterozygosity in the gerreids studied was caused by the first factor. However, low heterozygosity values have been correlated also with overfishing (Rivalta et al., 1987), this factor could explain the low values observed in *Eugerres*, which are fished in Puerto Progreso, and *Diapterus*, a fishery by-catch in Port Charlotte, but not in *Eucinostomus*, a small-size species with almost no fishing pressure in Puerto Progreso.

None of the allozyme systems was expressed by duplicate suggesting that polyploidy was not a factor in gerreid evolution. This result agrees with the cytogenetic

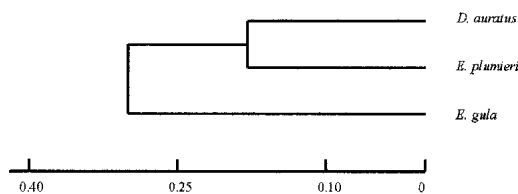


FIG. 1. Genetic similarity among gerreid taxa based on allozyme analysis.

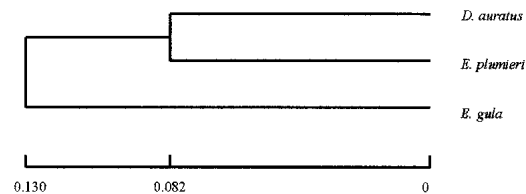


FIG. 2. The mtDNA sequence divergence between 3 gerreids.

TABLE 5. Size of mtDNA fragments produced with endonucleases Hae III, Hind III, and Taq I.

<i>E. gula</i>			<i>E. plumieri</i>			<i>D. auratus</i>		
Hae III	Hind III	Taq I	Hae III	Hind III	Taq I	Hae III	Hind III	Taq I
6.10	5.66	12.40	6.60	9.10	12.40	5.66	9.10	9.18
3.70	4.94	4.44	4.70	4.94	3.22	4.70	3.36	4.44
3.10	3.30	1.01	3.40	2.82	1.01	3.70	2.30	3.22
2.92	2.38		2.20		0.36	2.92	1.66	
0.62					0.21		0.65	
0.35								
16.79	16.28	17.85	16.90	16.86	17.20	16.98	17.07	16.86
MW = 16.97 kb			MW = 16.98 kb			MW = 16.96 kb		

analysis of *Eucinostomus* and *Eugerres* (Ruiz-Carus and Uribe-Alcocer, in press).

The analysis of 10 allozyme systems identified 3 protein-coding loci with diagnostic value for taxonomic identification. These are: GPI-1 muscle, LDH eye, and MDH liver. Allozyme polymorphism allowed separation of adult gerreids and could be used as genetic markers. The application of these markers to larval and juvenile specimens could be limited because the ontogenetic expression of these protein-coding loci is unknown.

The topology of the dendrogram recovered with allozymes concurs with Espinosa et al. (1993) study of serum proteins. The genetic differences between *E. plumieri* and *D. auratus* substantiate the separation of these genera based on morphology (Fisher, 1978; Deckert and Greenfield, 1987; Cervigon, 1993; Eschmeyer, 1998).

The mojarras mtDNA size in basepairs (bp) is within the range reported in other fishes (Berg and Ferris, 1984; Bird et al., 1986; Zardoya and Meyer, 1997). The small differences in total molecular weight obtained with different endonucleases (Table 5) are explained by the difficulty to detect fragments  $\leq 60$  bp. The small differences in mtDNA size were considered to be a by-product of the technique. In contrast, small differences in some taxa (e.g., *Amia calva*: Amiiformes) were due to actual mtDNA size polymorphism and heteroplasma (Bermingham et al. 1986).

The mtDNA analysis recovered the same topology as the allozyme analysis did. However, it should be clear that in in-

tensely studied groups (e.g., salmonids) mtDNA has shown different rate of evolution for each taxon (Gyllensten and Wilson, 1987), suggesting that divergence data should be used with caution. The use of divergence data outside the biological context might predispose to error when comparing species phylogenetically close but with different evolutionary history (Shed'ko, 1991). Mitochondrial DNA data are preliminary considering that only 172 bp were identified. This number represents only 0.003 % of total mtDNA, and the divergence values (Fig. 2) indicated 8.5-19.3 % divergence; the age of separation was not estimated because of this imprecision. The topology of the mtDNA dendrogram coincides with the allozyme topology and confirms the systematic arrangement based on morphological characters (Fisher, 1978; Deckert and Greenfield, 1987; Cervigon, 1993; Eschmeyer, 1998).

Allozymes GPI-1 muscle, LDH-c eye, and MDH liver allowed taxonomic identification of *E. gula*, *E. plumieri* and *D. auratus*. Genetic distance and genetic similarity indicated that *E. plumieri* and *D. auratus* are phylogenetically closer than any of the combinations between them and *E. gula*. The average mtDNA size was similar between the gerreids studied and mtDNA profiles allowed separation of the three taxa. The dendrograms of genetic similarity and mtDNA divergence had the same topology. *Eugerres* and *Diapterus* were clearly separated within the dendrograms; thus confirming the separation of these taxa at the generic level. Allozyme and mtDNA

TABLE 6. Total number of mtDNA restriction fragments, and number of fragments shared between gerreid pairs.

Enzyme	Total number of fragments			Number of fragments shared <sup>1</sup>		
	g	p	a	g/p	g/a	p/a
Hae III	6	4	4	0	2	1
Hind III	4	3	5	1	0	1
Taq I	3	5	3	2	1	1
Total	13	12	12	3	3	3

<sup>1</sup>g = *E. gula*, p = *E. plumieri*, a = *D. auratus*

analyses support the common taxonomic practice of grouping the mojarras with serrated preoperculum.

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