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Relationships among four genera of mojarras (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percomorph fishes

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A phylogenetic study of the percoid family Gerreidae at both lower and higher taxonomic levels is presented based on DNA sequence data of four genes: mitochondrial 12S and 16S, and nuclear genes rhodopsin and recombination activating gene 1 (RAG1). The taxonomic sampling includes four genera of Gerreidae from the western Atlantic, 39 additional percomorph representatives and two outgroups. Phylogenetic results confirm the monophyly of the Gerreidae and suggest that the family is divided into two sub-groups (*Diapterus auratus* plus *Eugerres plumieri* and *Eucinostomus gula* plus *Gerres cinereus*), which correspond to two previously defined taxonomic assemblages characterized by the shape of the preoperculum. Gerreids are placed at an intermediate position in the percomorph tree between two basal clades (L and Q) and a terminal clade N (grouping tetraodontiforms, acanthuroids, lophiiforms, caproids and several percoids). In addition, topology tests indicate that two traditional assemblages, Labroidei (seven representatives sampled) and Percoidei (22 representatives sampled) are not natural groups. Labrids and scarids appear to be more closely related to gerreids and to the members of clade N than to any other basal percomorphs, including their labroid 'allies' sampled in this study, Embiotocidae, Pomacentridae and Cichlidae, which are all nested within clade Q that also includes atherinomorphs, mugiliforms and Chandidae. The percoid taxa included in this study are widely distributed among various percomorph lineages. The percomorph phylogeny obtained is highly congruent with results from recent molecular studies.

Key words: Gerreidae; Labroidei; mt-ribosomal gene; nuclear protein-coding gene; Percoidei; percomorph phylogeny.

INTRODUCTION

Gerreids or mojarras comprise small- to medium-sized, strongly compressed fishes characterized by a pointed snout and a highly protrusible mouth. They occur over muddy and sandy bottoms in estuaries, hypersaline lagoons, and occasionally in fresh water in tropical and subtropical shallow coastal habitats

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(Cervigon, 1993; Nelson, 1994). Currently, six genera are recognized in the family: *Diapterus*, *Eucinostomus*, *Eugerres*, *Gerres*, *Parequula* and *Pentaprion*, with a total of 50 species known globally (Eschmeyer, 1998; FishBase, http://www.fishbase.org). The latter two genera are monotypic and occur in the eastern Indian Ocean and Indo-West Pacific, respectively. The mojarras of the western Atlantic comprise species within the first four genera and *c.* 13 species. They are a major component of the estuarine ichthyofauna, as well as an important constituent of subsistence fisheries in some localities.

The placement of gerreids among percomorph fishes has varied over time according to different authors studying gerreid affinities to perciform lineages. The perceived closer affinity to perch-like fishes such as Percidae, Sparoidea (Sparidae and its allies) and Haemulidae and its allies (Günther, 1880; Stiassny, 1981; Johnson, 1984; Nelson, 1984; Rosen & Patterson, 1990) prompted Nelson (1994) to place the family Gerreidae within the Percoidei, the largest and most diversified of the perciform suborders, encompassing 71 families and *c.* 3000 species. This suborder comprises all perch-like fishes but is most probably a polyphyletic group (Johnson & Patterson, 1993) with unsettled classification. Currently, there is no evidence to support monophyly of Percoidei or even Perciformes, and the 18 or so proposed suborders of Perciformes remain with uncertain affinities. In addition to this proposed classification of Gerreidae, support for a close relationship between Labroidei and gerreids has been suggested based on gill-arch anatomy (Stiassny, 1981; Rosen & Patterson, 1990). It should also be noted that before 1880, Gerreidae was consistently placed within the 'pharyngognathi', which consisted of the currently recognized labroid fishes, *i.e.* labrids, scarids, odacids, pomacentrids, embiotocids and cichlids (Rosen & Patterson, 1990). However, the evidence to support this assumption was based on a relatively small number of morphological characters.

The taxonomy within the family Gerreidae is also under considerable debate, primarily due to morphological plasticity in some taxa and to uncertain definitions of valid genera. Seven nominal genera have been proposed for Gerreidae in the western Atlantic Ocean: *Diapterus, Eucinostomus, Eugerres, Gerres, Lepidochir*, *Moharra* and *Ulaema*, but several authors have disputed the validity and limits of these genera. Deckert & Greenfield (1987) placed *Moharra* Poey 1875 as a junior synonym of *Diapterus*. Curran (1942) described the monotypic genus *Lepidochir* in his doctoral dissertation, but his study has not been published, consequently *Lepidochir havana* is not a valid taxon according to the ICZN (1999), and should be accepted as *Eucinostomus havana* (Nichols 1912). Also, the monotypic *Ulaema lefroyi* was placed within *Eucinostomus* (Castro-Aguirre *et al.*, 1999), leaving four recognized genera: *Eucinostomus* Baird & Girard 1855, *Eugerres* Jordan & Evermann 1927, *Diapterus* Ranzani 1842 and *Gerres* Quoy & Gaimard 1824 (Eschmeyer, 1998). Recently, mojarras with a serrated preoperculum were assigned to *Diapterus* (Robins *et al.*, 1991) or to a combination of *Diapterus* and *Eugerres* (Fisher, 1978; Cervigon, 1993; Castro-Aguirre *et al.*, 1999), while those with a smooth preoperculum were assigned to *Eucinostomus* and *Gerres* (Deckert & Greenfield, 1987; Cervigon, 1993; Claro, 1994; Hoese & Moore, 1998; Castro-Aguirre *et al.*, 1999) or simply to *Gerres* (Andreata, 1989).

More recent attempts to study gerreid affinities were based on allozymes (Espinosa *et al.*, 1993), and allozymes and restriction profiles of mtDNA

[restriction fragment length polymorphism (RFLP)] (Ruiz-Carus & Uribe-Alcocer, 2003). A dendrogram of genetic similarity generated by unweighted pair group method with arithmetic mean on the mtDNA RFLP patterns (Ruiz-Carus & Uribe-Alcocer, 2003) grouped *Diapterus* plus *Eugerres* and *Eucinostomus* plus *Gerres*, in agreement with the taxonomic assemblages based on the shape of the preoperculum. In the present study, nucleotide sequences of nuclear and mitochondrial genes are collected and analysed to test (1) the evolutionary relationships of *Gerres cinereus* Walbaum, 1792, *Eucinostomus gula* (Quoy & Gaimard 1824), *Eugerres plumieri* (Cuvier 1830) and *Diapterus auratus* Ranzani 1840; and (2) the placement of Gerreidae among percomorphs.

MATERIALS AND METHODS

SAMPLE COLLECTION

Adult gerreids, lutjanids, sciaenids and haemulids were captured in the vicinity of Tequesta, Florida (27·10206° N; 80·09810° W). A 183 m × 3·0 m centre-bag seine (38·1 mm stretched mesh with a 3·0 m × 3·0 m bag) was used. The seine was deployed from the stern of a mullet skiff in a semicircle and hauled to shore. Taxonomic identification followed Deckert & Greenfield (1987) and Matheson & McEachran (1984). Fishes were killed on site and tissue samples were dissected and stored in 95% ethanol. Specimens were fixed in 10% buffered formalin and preserved in 50% isopropanol. The preserved specimens were deposited in the FWC-Fish and Wildlife Research Institute's Ichthyology collection (FSBC). Tissue samples and DNA sequences for additional species used in high-level taxonomy were obtained from several sources (Ortí laboratory and W.J.C. tissue collection) and GenBank (Table I).

LABORATORY MOLECULAR WORK

Tissue extraction was performed using Qiagen DNeasy extraction kit (Qiagen, Valencia, CA, U.S.A.), according to the manufacturer's instructions. DNA amplification was conducted by polymerase chain reaction (PCR) (Mullis & Faloona, 1987; Saiki *et al.*, 1988) for fragments of the mtDNA 12S and 16S ribosomal genes, and for exon 3 of recombination activating gene 1 (RAG1) and a fragment of rhodopsin. Primers used in this study were published by Kocher *et al.* (1989) for 12S, by Palumbi (1996) for 16S, by López *et al.* for RAG1, and by Chen *et al.* (2003) for rhodopsin. One new reverse primer (R1-4061R) was designed to obtain RAG1 sequences of *E. plumieri* and *G. cinereus*. The sequence of this primer is: 5′-AATACTTGGAGGTGTAGAGCCAGT-3′. Conditions for amplification were as follows: 0·2 units of *Taq* polymerase (Gibco, Life Technologies Inc., Gaithersburg, MD, USA), 1× reaction buffer (Gibco), 3 mM of $MgCl_2$, 0·2 mM of each dNTP, 0·4 mM of each primer and 25–50 ng of genomic DNA in a 25 μl final reaction volume. A high fidelity Takara *Ex Taq* (0·625 units) (TAKARA Bio Inc., Otsu, Japan) was used to amplify RAG1 exon 3 fragment. Thermocycler conditions for PCR were: initial denaturing step at 95° C for 4 min followed by 35 cycles of 95° C (for 45 s), annealing melting temperature (Tm) (for 30 s), and 72° C (for 1–1·5 min depending on size of fragments), and then a final extension step of 72° C (for 7 min). Tm was 55, 55, 58 and 53° C for 12S, 16S, rhodopsin and RAG1, respectively. PCR cleanup procedure followed the shrimp alkaline phosphatase (SAP)/*Exo*I protocol: 1 μl of SAP (1 unit) and 0·2 μl of ExonucleaseI were added to 10 μl PCR product, and incubated at 37° C for 30 min, and then at 80° C for 15 min. BigDye (v3.0; Applied Biosystems, Foster City, CA, USA) chemistry was used for direct sequencing of the purified PCR products and the sequences were determined with a BaseStation 5100 analyzer (MJ Research, Waltham, MA, USA). A few sequences were determined by Macrogen Inc. (Seoul, South Korea) using an ABI 3730xl analyzer (Applied Biosystems).

TABLE I. Taxa included in this study

Order/suborder	Family	Taxon	GenBank accession number			
			12S	16S	Rhodopsin	RAG1
Outgroups						
Beryciformes						
Trachichthyoidei	Trachichthyidae	*Hoplostethus mediterraneus*	AY141335	AY141405	AY141264	EF095635*
Berycoidei	Berycidae	*Beryx splendens*	AY141336	AY141406	AY141265	EF095636*
Percomorpha						
Lophiiformes						
Lophioidei	Lophiidae	*Lophius budegassa*	EF095552*	EF095580*	EF095608*	EF095637*
'Zeiformes'						
Caproidei	Caproidae	*Capros aper*	EF095553*	EF095581*	AY141262	EF095638*
Mugiliformes	Mugilidae	*Mugil cephalus*	EF095554*	EF095582*	EF095609*	EF095639*
Atheriniformes						
Bedotioidei	Bedotiidae	*Bedotia geayi*	AY141339	AY141409	AY141267	EF095640*
Beloniformes						
Adrianichthyoidei	Adrianichthyidae	*Oryzias latipes*	EF095555*	EF095583*	AB001606	EF095641*
Scorpaeniformes						
Scorpaenoidei	Scorpaenidae	*Scorpaena onaria*	AY141364	AY141434	AY141288	EF095642*
Tetraodontiformes						
Tetraodontoidei	Molidae	*Mola mola*	AY141361	AY141431	AY141286	EF095643*
		Takifugu rubripes	AJ421455	AJ421455	AF201471	AF108420
Pleuronectiformes						
Pleuronectoidei	Soleidae	*Solea solea*	EF095556*	EF095584*	Y18672	EF095644*
	Achiridae	*Trinectes maculatus*	AY430282	AY430244	EF095610*	AY430224
Perciformes						
Percoidei	Serranidae	*Holanthias chrysostictus*	AY141366	AY141436	AY141290	EF095645*
	Chandidae	*Parambassis ranga*	EF095557*	EF095585*	EF095611*	EF095646*
		Parambassis wolffii	EF095558*	EF095586*	EF095612*	EF095647*
	Centropomidae	*Lates calcarifer*	AY141371	AY141441	AY141294	EF095648*
		Centropomus sp.	EF095559*	EF095587*	EF095613*	EF095649*

TABLE I. Continued

Order/suborder	Family	Taxon	GenBank accession number			
			12S	16S	Rhodopsin	RAG1
	Moronidae	*Lateolabrax japonicus*	AY141369	AY141439	AY141293	EF095650*
		Dicentrarchus labrax	AY141370	AY141440	Y18673	EF095651*
	Toxotidae	*Toxotes chatareus*	EF095560*	EF095588*	EF095614*	EF095652*
	Coryphaenidae	*Coryphaena sp.*	EF095561*	EF095589*	EF095615*	EF095653*
	Carangidae	*Parastromateus niger*	EF095562*	EF095590*	EF095616*	EF095654*
	Chaetodontidae	*Chaetodon semilarvatus*	EF095563*	EF095591*	AY368312	EF095655*
	Drepaneidae	*Drepane africana*	EF095564*	EF095592*	AY141321	EF095656*
	Sparidae	*Sparus aurata*	EF095565*	EF095593*	Y18665	EF095657*
	Mullidae	*Mullus surmuletus*	EF095566*	EF095594*	EF095617*	EF095658*
	Menidae	*Mene maculata*	AY141390	AY141460	AY141316	EF095659*
	Sciaenidae	*Cynoscion regalis*	EF095567*	EF095595*	EF095618*	EF095660*
	Haemulidae	*Haemulon aurolineatum*	EF095568*	EF095596*	EF095619*	EF095661*
	Lutjanidae	*Lutjanus analis*	EF095569*	EF095597*	EF095620*	EF095662*
	Gerreidae	*Eucinostomus gula*	EF095570*	EF095598*	EF095621*	EF095663*
		Diapterus auratus	EF095571*	EF095599*	EF095622*	EF095664*
		Eugerres plumieri	EF095572*	EF095600*	EF095623*	EF095665*
		Gerres cinereus	EF095573*	EF095601*	EF095624*	EF095666*
Elassomatoidei	Elassomatidae	*Elassoma zonatum*	EF095574*	EF095602*	EF095625*	EF095667*
Acanthuroiei	Scatophagidae	*Scatophagus argus*	AF055598	AF055619	EF095626*	EF095668*
Labroidei	Labridae	*Labrus bergylta*	AY141392	AY141462	EF095627*	EF095669*
	Embiotocidae	*Embiotoca jacksoni*	AY279573	AY279676	EF095628*	EF095670*
	Cichlidae	*Astronotus ocellatus*	EF095575*	EF095603*	EF095629*	EF095671*
		Etroplus maculatus	EF095576*	EF095604*	EF095630*	EF095672*
	Pomacentridae	*Pomacentrus pavo*	EF095577*	EF095605*	EF095631*	EF095673*
		Dascyllus aruanus	AF081228	AF119402	EF095632*	EF095674*
	Scaridae	*Scarus psittacus*	EF095578*	EF095606*	EF095633*	EF095675*
Scombroidei	Scombridae	*Scomberomorus commerson*	EF095579*	EF095607*	EF095634*	EF095676*
Stromateoidei	Centrolophidae	*Psenopsis anomala*	AY141384	AY141454	AY141310	EF095677*

Classification is according to Nelson (1994).

Accession numbers with an asterisk are sequences obtained in this study.

DATA SETS

The DNA sequences were edited and managed with BioEdit v7.0 (Hall, 1999) and Se-Al v2.0a11 (Rambaut, 1996). Data set was constructed based on the four gene partitions: 12S (*c.* 345 bp), 16S (*c.* 376 bp), rhodopsin (759 bp) and RAG1 exon 3 (1473 bp). The latter nuclear marker is now frequently employed for higher level systematic studies of fishes (Holcroft, 2004; López *et al.*, 2004; Quenouille *et al.*, 2004; Rüber *et al.*, 2004; Holcroft, 2005) and tetrapods (Groth & Barrowclough, 1999; Waddell & Shelley, 2003; San Mauro *et al.*, 2004; Steppan *et al.*, 2004; Krenz *et al.*, 2005).

Taxonomic sampling was composed of the samples from four gerreid species, representative percoid families, labroid families (wrasses, parrotfishes, damselfishes, surfperches and cichlids) and several selected backbone taxa of percomorphs, according to the phylogenies established in Chen *et al.* (2003) and Miya *et al.* (2003) plus two beryciforms (outgroups) with a total of 45 taxa of which 22 belong to 16 individual percoid families (Table I).

PHYLOGENETIC ANALYSES

Sequences were initially aligned with Clustal X (Thompson *et al.*, 1997) and then adjusted manually based on the inferred amino acid translation or secondary structure of ribosomal DNA. The secondary structure of teleost 12S and 16S were published in Ortí *et al.* (1996), Waters *et al.* (2000) and Wang & Lee (2002). The regions where the amount of variation was very high and the resulting alignment would likely contain invalid assertions of homology, *i.e.* large insertion/deletion segments showing high dissimilarity in sequence length, were discarded from the phylogenetic analyses. Secondary structure models and aligned sequence matrices are available upon request.

Phylogenetic analyses were based on a partitioned Bayesian approach as implemented in MrBayes parallel version v3.1.1 (Huelsenbeck & Ronquist, 2001). Maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP* version 4.0b10 (Swofford, 2002) were also used to compare results. Optimal trees were obtained by heuristic searches with random stepwise addition sequences followed by TBR (tree bisection-reconnection) swapping, for 100 and 10 replications each, for MP and ML analysis, respectively (Swofford, 2002). Likelihood ratio tests (Goldman, 1993), as implemented in MODELTEST 3.06 (Posada & Crandall, 1998), were used to choose models for model-based methods. The substitution model selected for ML was GTR+G+I. Partitioned Bayesian phylogenetic analysis was performed with implementation of a more complex and realistic model by assigning separate properties to each gene and each codon partition (suggested by MODELTEST): the GTR+G+I model (Yang, 1994) for 12S, 16S and the first and second codon positions of RAG1; the HKY+G+I model (Hasegawa *et al.*, 1985) for the first codon position of rhodopsin; the HKY+G model for the third codon positions of RAG1 and rhodopsin; and the F81+G+I model (Felsenstein, 1981) for the second codon position of rhodopsin. The parameters for running MrBayes were set as follows: 'lset nst = 6' (GTR) or 'lset nst = 2' (HKY), or 'lset nst = 1' (F81), 'rates = invgamma' (G+I), or 'rates = gamma' (G), 'unlink' (unlinking of model parameters across data partitions), and 'prset ratepr = variable' (rate multiplier variable across data partitions). Four independent Markov chain Monte Carlo (MCMC) chains were performed with 3 000 000 replicates, sampling one tree per 100 replicates for each run. This procedure was repeated until stationary log-likelihoods were observed. Initial trees with non-stationary log-likelihood values as part of a burn-in procedure were discarded. The remaining trees from two independent runs that resulted in convergent log-likelihood scores were used to construct a 50% majority rule consensus tree. The resulting *a posteriori* probabilities were considered a measure of node support. In addition, node support was also assessed using the bootstrap procedure (Felsenstein, 1985) under the MP criterion, based on 1000 pseudoreplicates of heuristic searches, as described above.

A test of homogeneity of base frequencies across taxa was conducted for each gene and codon position separately using the chi-square test implemented in PAUP*. The deviant taxa were subsequently identified by the chi-square test as implemented in Puzzle 4.02 (Strimmer & von Haeseler, 1996).

Previous hypotheses for the monophyly of Percoidei (Nelson, 1994), Labroidei (Kaufman & Liem, 1982; Stiassny & Jensen, 1987), Perciformes (Nelson, 1994) and Pharyngognathi, *i.e.* labroids plus gerreids (Gill, 1872) were tested using the approach proposed by Templeton (1983) (TP), and by Shimodaira & Hasegawa (1999) (S-H) for MP and ML analysis, respectively, as implemented in PAUP*. Five constrained analyses corresponding to previous hypotheses (Table II) were conducted. The tree scores (tree length for MP analysis, log likelihood for ML analysis) resulting from these constrained analyses were then compared with the tree score of the best trees under these criteria. The differences in tree length and likelihood scores between topologies (the best and constrained ones) were statistically evaluated *via* a non-parametric method (for TP) and the resampling approach (resample estimated log-likelihood) with 10 000 bootstrap replications (for the S-H test).

RESULTS

CHARACTERISTICS OF SEQUENCE DATA

DNA sequences for the four gene fragments were obtained directly from tissues or from GenBank for a common set of 45 species. A total of 2953 aligned nucleotide characters were collected (345 bp for 12S, 376 bp for 16S, 759 bp for rhodopsin and 1473 bp for RAG1) among which 1203 parsimony-informative characters were found. All sequences of rhodopsin and RAG1 (exon 3) contained a single open reading frame. Three taxa (*Mullus surmuletus* Linnaeus 1758, *Takifugu rubripes* (Temminck & Schlegel 1850) and *Parastromateus niger* (Bloch 1795)) showed indels with one amino acid deletion at position 143 of the RAG1 fragment. No introns were found in the rhodopsin sequences collected, which is in agreement with the 'intron-less' hypothesis for this gene in ray-finned fishes except bichirs (Fitzgibbon *et al.*, 1995; Venkatesh *et al.*, 1999). Base composition stationarity could not be rejected based on chi-square tests performed on all sites for each gene partition ($P = 1$, 1, 1 and 0·987 for 12S, 16S, rhodopsin and RAG1, respectively). The null hypothesis was rejected only for the test on the third codon position of RAG1. Seven deviant taxa

TABLE II. Alternative hypothesis tests for the previously defined monophyletic groups

	Parsimony		Likelihood	
Hypothesis	Diff. L	TP test	Diff. lnL	S-H test
Perciformes[a]	86	<0·001*	248·093	<0·001*
Percoidei[a]	97	<0·001*	260·283	<0·001*
Labroidei 1[b]	41	0·0016*	92·514	0·0082*
Labroidei 2[c]	34	0·0270*	91·398	0·0089*
Pharyngognathi[d]	46	<0·001*	124·162	0·0038*

Statistical significant differences ($P < 0·05$) are indicated by asterisks.

Diff. L, length difference to the best (MP) tree; Diff. ln L, log-likelihood difference to the best (ML) tree; TP, Templeton; S-H, Shimodaira–Hasegawa.

[a]Monophyly of Perciformes and Percoidei according to the classification of Nelson (1994).

[b]Stiassny & Jensen (1987) hypothesis of labroid monophyly and intrarelationships, corresponding to '(Cichlidae, (Embiotocidae, (Pomacentridae, Labridae plus Scaridae)))'.

[c]Kaufman & Liem (1982) hypothesis of labroid monophyly and intrarelationships, corresponding to '(Pomacentridae, (Cichlidae, (Embiotocidae, Labridae plus Scaridae)))'.

[d]Pharyngognathi (labroids and gerreids) as defined by Gill (1872).

were detected; these were: Scatophagus, Etroplus, Astronotus, Mullus, Scorpaena, Sparus and Pomacentrus. These taxa had statistically significant lower (the first three taxa) or higher (the other four) guanine plus cytosine (GC) content than the mean value (0·63) on the third codon position of RAG1. When base composition varies significantly among taxa, all classical methods of phylogenetic construction tend to group sequences of similar nucleotide composition together, regardless of evolutionary history (Lockhart *et al.*, 1994). However, this artefact was unlikely to affect the consistency of the phylogenetic analysis because all aberrant taxa detected, except for two cichlid (with low GC), were placed at dispersed positions in the tree (Fig. 1). In addition, analysis that excluded third codon position sites of RAG1 resulted in trees with similar clustering patterns as those shown in Fig. 1.

PERCOMORPH PHYLOGENY

The results of ML analysis and the consensus tree of the partitioned Bayesian analysis are shown in Fig. 1. The topologies obtained from these analyses were almost identical, with the exceptions noted in the Fig. 1. MP analyses produced similar results with some differences particularly among taxa in group 'N' (Fig. 1). Long terminal branches and short internal branches characterized the phylogeny, reflecting either mutational saturation or the putative fast radiation of Percomorpha during Late Cretaceous (Benton, 1993; Patterson, 1993). The latter is most likely since absolute saturation tests (Philippe *et al.*, 1994) did not detect a diagnostic saturation plateau. These tests were performed on transitions and transversions for each gene and codon position separately. The results for the third codon position of rhodopsin and RAG1 exon 3 are shown in Fig. 2. Not surprisingly, deep branches were in general weakly supported (Fig. 1), but a few clades received relatively robust support. Most noteworthy were clade L and clade Q (Fig. 1). Clade L was previously reported in a study based on multiple nuclear and mitochondrial loci and includes Pleuronectiformes (flatfishes), Carangidae (jacks), Echeneidae (remoras), Sphyraenidae (barracudas), Menidae (moon fish), Polynemidae (threadfins) and Centropomidae (snooks) (Chen *et al.*, 2003). In this study, two additional percoid members, Toxotidae and Coryphaenidae were nested within clade L. The second clade (Q) contained Atherinomorpha, Mugiliformes, Pomacentridae (Labroidei), Cichlidae (Labroidei), Embiotocidae (Labroidei) and one of the percoid families sampled in this study—Chandidae. The topology of the rest of the tree was not strongly supported. The family Gerreidae was placed within this portion of the tree, at an intermediate position between early branching lineages such as stromateoids, scombrids, mullids, elassomatids, scorpaenids, serranids and a terminal clade containing tetraodontiforms, acanthuroids, lophiiforms and other percoid relatives. Finally, the representative taxa sampled from Percoidei, Labroidei and Perciformes did not form monophyletic groups. Topological tests for these hypotheses rejected the monophyly of these putative groups (Table II) based on the molecular data.

PHYLOGENY OF FOUR GENERA OF THE GERREIDAE FROM THE WESTERN ATLANTIC

The phylogenetic analysis of this study confirmed the monophyly of the Gerreidae. The results divided this family into two sub-groups, one containing

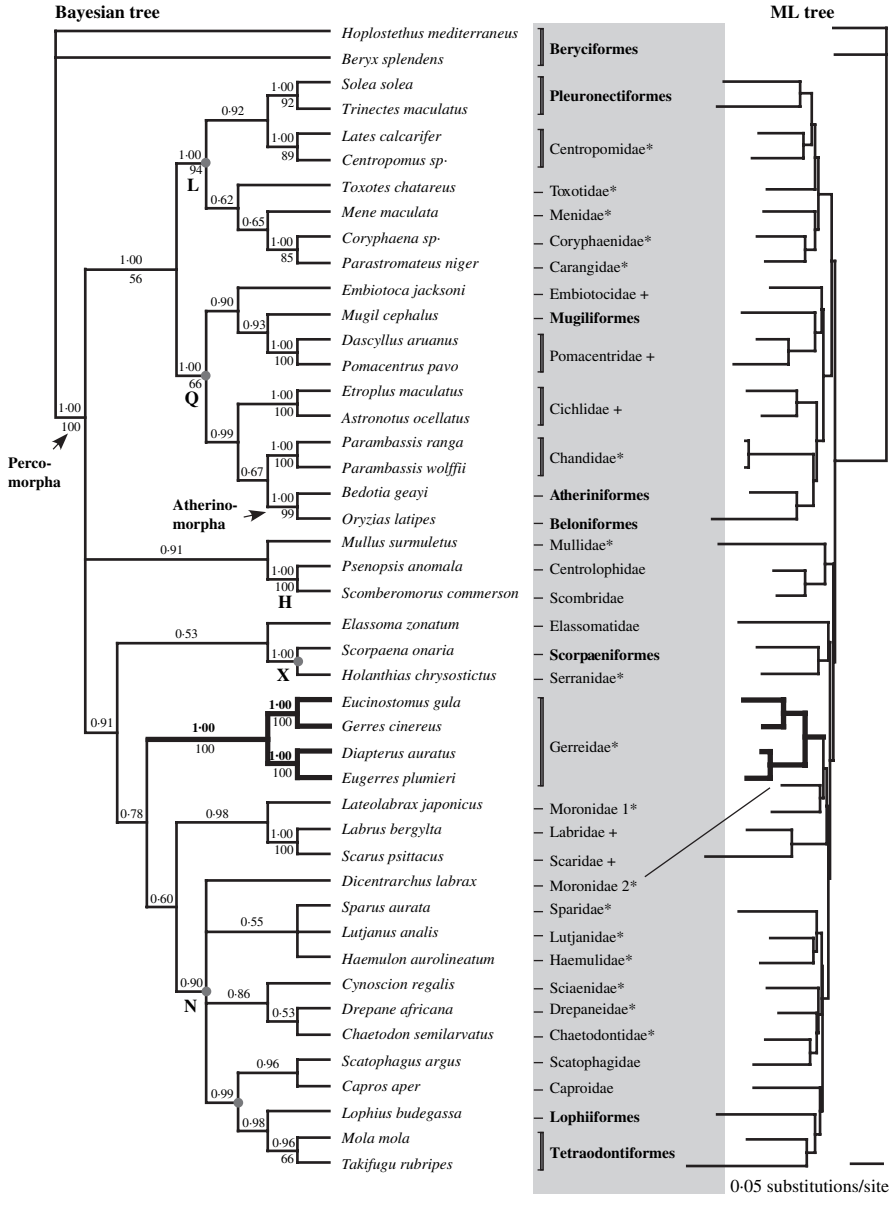

FIG. 1. Fifty per cent majority rule consensus tree of all post burn-in trees (58 233 trees) from partitioned Bayesian analyses on the left, and maximum likelihood (ML) on the right (ML score of 40 561·1444) obtained from the combined data set of 12S, 16S, rhodopsin and RAG1 gene partitions (2953 bp) depicting percomorph relationships. Gerreids are clades in bold lines. ML tree branch length is proportional to inferred character substitutions under GTR+G+I model. Numbers above the branches of topology at left represent Bayesian posterior probabilities. MP bootstraps are shown below the branches of topology at left. Values below 50% are not shown. Percoid and labroid families (Perciformes) are indicated with a star and a plus sign, respectively. Clades L, Q, X, H and N are consistent with the molecular phylogeny of acanthomorphs from Chen *et al.* (2003) and Dettai & Lecointre (2005). The dark grey points on the node are clades in agreement with the phylogeny of Miya *et al.* (2003) based on whole mitochondrial genomic data.

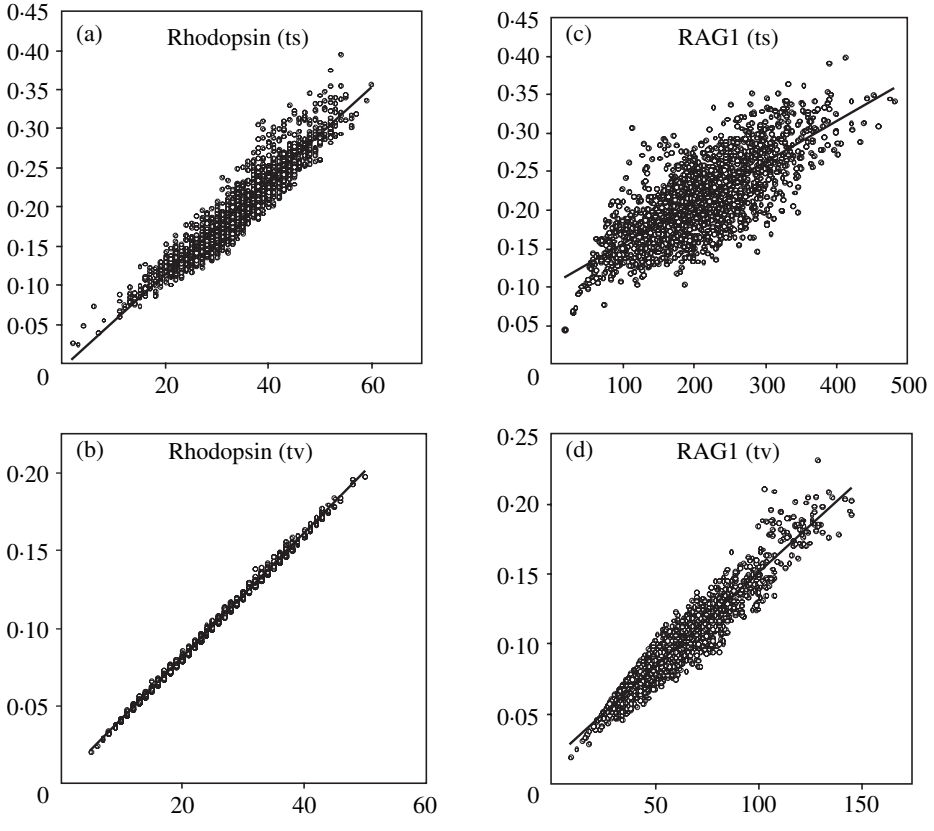

FIG. 2. Absolute saturation tests (Philippe *et al.*, 1994) for the nuclear genes used in the phylogenetic analysis. Plots show transitions (ts) at third codon positions of rhodopsin (A) and RAG1 (C) and transversions (tv) at third codon positions of rhodopsin (B) and RAG1 (D). *X*-axis, number of substitutions among all pairs of terminals inferred from the MP tree; *Y*-axis, pair-wise sequence differences (fraction of sites that differ between two sequences or 'p-distance').

E. gula plus *G. cinereus* and the other with *D. auratus* plus *E. plumieri*. The monophyly of the family and both of the sub-groups were highly supported by posterior probabilities and bootstrap values (Fig. 1). Sequence divergences (ML distance based on the GTR+G+I model) ranged from 0·04291 to 0·14895 within the family, and from 0·13250 to 0·26824 between gerreids and other percomorph taxa. The divergence value between *E. gula* and *G. cinereus* was 0·08295, while that between *D. auratus* and *E. plumieri* was 0·04291.

DISCUSSION

Recent higher level molecular systematic studies of higher teleost fishes (Chen *et al.*, 2003; Miya *et al.*, 2003; Holcroft, 2004; Smith & Wheeler, 2004; Dettai & Lecointre, 2005) produced somewhat unexpected results, when compared with traditional hypotheses based on morphology (Lauder & Liem, 1983; Stiassny & Moore, 1992; Johnson & Patterson, 1993), that need further testing by more

intensive taxonomic sampling and additional independent molecular markers, especially for some key groups such as the Percoidei, Labroidei and Perciformes.

In the present study, the authors attempted to assess the relationships of Gerreidae—currently classified within the Percoidei, based on 2953 aligned nucleotide characters from two nuclear and two mitochondrial genes. The taxa included in this analysis include the potential candidates proposed on the basis of morphology to have closer affinity to gerreids, and also additional percomorph lineages, given that Perciformes and its numerous subdivisions are probably polyphyletic (*e.g.* Labroidei, Percoidei). A reasonable assessment of the phylogenetic position of Gerreidae may not be obtained without establishing a backbone percomorph phylogeny covering a diverse taxonomic spectrum.

The phylogenetic results show that Perciformes, Percoidei and Labroidei are polyphyletic groups (Fig. 1 and Table II), and provide no support for previously established morphological hypotheses of evolutionary relationships among acanthomorphs (Lauder & Liem, 1983; Stiassny & Moore, 1992; Johnson & Patterson, 1993). The concept of Smegmamorpha (Johnson & Patterson, 1993), a group that includes Synbranchiformes (spiny and swamp eels), Mugiloidei (mullets), Elassomatidae (pygmy sunfishes), Gasterosteiformes (pipefishes and sticklebacks) and Atherinomorpha (silversides and relatives) and the 'unnamed' sister group of the Smegmamorpha, which groups Dactylopteriformes (flying gurnards), Scorpaeniformes (mail-cheeked fishes), Perciformes, Tetraodontiformes (pufferfishes) and Pleuronectiformes (flatfishes) is not supported by the molecular data. In contrast to this hypothesis, the molecular phylogeny in this study generates two robust clades, clade L and clade Q (Fig. 1), which include primarily pleuronectiforms and atherinomorphs, respectively. Gerreids, and a number of diverse 'perciform' lineages, elassomatids, scorpaeniforms, lophiiforms and notably tetraodontiforms are placed in uncertain positions but clearly outside of clades L and Q.

The molecular tree shown in Fig. 1 is highly congruent with the results from other molecular systematic studies with similar taxonomic coverage. These studies were based on sequences of protein-coding genes from the complete mitochondrial genome (Miya *et al.*, 2003), two mitochondrial ribosomal genes plus two nuclear genes (28S and rhodopsin) (Chen *et al.*, 2003) and the nuclear mixed lineage leukaemia-like gene (Dettai & Lecointre, 2005). Clade L was initially proposed by Chen *et al.* (2003), implying a 'percoid origin' of Pleuronectiformes (Chapleau, 1993). This clade also was recovered by Miya *et al.* (2003) and confirmed by Dettai & Lecointre (2005). Although taxonomic components of this clade are slightly different among the different molecular studies, they all suggest close affinities among Pleuronectiformes and several perciform families, including Carangidae, Echeneidae, Coryphaenidae [these three belong to the percoid superfamily, Carangoidea, defined by Johnson (1984)], Centropomidae, Toxotidae, Menidae, Polynemidae and Sphyraenidae (Scombroidei).

Clade Q was previously reported by Dettai & Lecointre (2005), grouping Cichlidae (Labroidei), Atherinomorpha, Mugiliformes and the clade D of Chen *et al.* (2003), consisting of Blennioidei and Gobiesocidae. This same clade and its affinities also have been supported by mitogenomic evidence (Miya *et al.*, 2003). Unfortunately, cichlids were not included in Miya's analysis. In the present study, the authors included representatives of most labroid lineages to

explicitly test the monophyly of Labroidei for the first time and to infer relationships among labroids and gerreids. The results showed that three labroid families, Pomacentridae, Embiotocidae and Cichlidae, are nested in clade Q. In addition, a percoid taxon, Chandidae (Asiatic glassfishes), also is present in this clade. However, labrids (wrasses) and certainly scarids (parrotfishes) are not included (Fig. 1). The clade grouping labrids and scarids was highly supported (Fig. 1) and its monophyly has been recognized in recent molecular studies (Streelman *et al.*, 2002; Westneat & Alfaro, 2005). The closer affinity between cichlids and atherinomorphs, rather than with other typical percomorphs (*e.g.* Tetraodontiformes, a member of clade N), also has been demonstrated by a phylogenomic analysis of 20 nuclear protein-coding genes (Chen *et al.*, 2004). In contrast, there is no morphological evidence published to support clade Q. Gosline (1968, 1971) recognized a close relationship among mugiloids, atherinoids, sphyraenids and polynemids based on the absence of an attachment of the pelvic girdle to the cleithra, but cichlids and pomacentrids also lack this particular character.

Three additional clades (X, H and N; Fig. 1) have been previously identified by molecular studies (Chen *et al.*, 2003; Miya *et al.*, 2003; Dettai & Lecointre, 2004; Holcroft, 2004; Smith & Wheeler, 2004; Dettai & Lecointre, 2005; Holcroft, 2005). According to Dettai & Lecointre (2004), clade X (*Scorpaena* plus *Holanthias* in this study) contains mainly 'scorpaeniforms' (rockfishes, gurnards and relatives) and also diverse perciform groups; zoarcoids (eelpouts), gasterosteoids (sticklebacks), serranids (sea basses), percids (freshwater perches), trachinids (weeverfishes) and notothenioids (Antarctic acanthomorphs). In a parallel study, Smith & Wheeler (2004) carried out a large-scale phylogenetic analysis of mitochondrial and nuclear DNA sequences of 105 acanthomorphs (sampling exclusively all lineages of the Scorpaeniformes) to test scorpaeniform monophyly. They found that the traditional Scorpaeniformes is not monophyletic. Clade H shows scombrids (mackerels and tunas) and stromatoids related to one another (Chen *et al.*, 2003). Finally, clade N in Dettai & Lecointre (2005) grouped drepaneids, chaetodontids, pomacanthids, 'acanthuroids', caproids, lophiiforms and tetraodontiforms. This grouping is consistent with the terminal clade in the acanthomorph tree of Holcroft (2004) based on RAG1-exon 3 nucleotide sequences. Holcroft (2004) provided the first report on sister-group relationships of Tetraodontiformes using molecular data. The results of this study corroborate these findings and suggest that several other percoids may be included in clade N: sparids, *Dicentrarchus*, lutjanids, sciaenids and haemulids. Mok & Chang (1986) suggested affinities between caproids and tetraodontiforms based on the articulation between the pelvic spine and the pelvic bone. Coincidently, this apomorphic character can also be found in chaetodontids, pomacanthids, scatophagids, pentacerotids, siganids and acanthurids (Holcroft, 2004). However, given that clade N may contain additional taxa belonging to Percoidei such as lutjanids, sciaenids, sparids, and haemulids, the distribution of the morphological synapomorphies and the taxonomic components of this group should be investigated further.

Regarding interrelationships among Gerreidae and Labroidei, Rosen & Patterson (1990) published a careful survey of a wide range of percomorphs for the anatomy of buccal and pharyngeal jaws, basicranial specializations

associated with pharyngeal jaws and the palatal musculature attaching to the prootic and parasphenoid bones. They revived the concept of the Pharyngognathi, but their grouping not only included classical pharyngognath fishes (labroids) but also sparoids (Sparidae and its relatives), haemuloids (Haemulidae and its relatives), kyphosids, scorpidids, girellids and notably gerreids. None of the molecular studies mentioned above included gerreids. None of them presented a single analysis representative from the major lineages of Labroidei and/or Percoidei. Although the results should be considered with caution, the taxonomic sampling in this study is relevant to address the issue, including seven labroids and 22 percoids. This study indicates that Labroidei is not monophyletic (Fig. 1 and Table II), thus rejecting the hypothesis of evolutionary affinity of gerreids to the entire Labroidei (Gill, 1872). In fact, gerreids and the labri-scarid clade were placed at an intermediate position between the terminal clade N and more basal clades L and Q. From a morphological perspective, based on the survey of Rosen & Patterson (1990), some aspects of the buccal anatomy of percomorphs seem consistent with the phylogenetic results of this study. The structure of the maxillary crest and the dorsal process (and therefore, the intervening groove for autopalatine) are relatively small in cichlids, embiotocids, pomacentrids and other 'primitive' perciforms such as serranids and centrarchids. In contrast, the maxillary anatomy is very well developed in labrids, gerreids, Haemuloidea, Sparoidea, some of squamipinnes (for instance, *Chaetodon*, *Pomacanthus*, *Scatophagus*, *Drepane*) and tetraodontiforms (Johnson, 1980; Rosen, 1984; Rosen & Patterson, 1990). However, the derived condition is not present in a few representatives from clade N: lutjanids, caproids and acanthuroids (Johnson, 1980; Stiassny, 1986; Tyler *et al.*, 1989; Rosen & Patterson, 1990).

Finally, the phylogenetic results among the four genera of western Atlantic gerreids are congruent with previous allozyme and RFLP mtDNA analyses (Espinosa *et al.*, 1993; Ruiz-Carus & Uribe-Alcocer, 2003). The four gerreids included in this study split into two clades, *D. auratus* plus *E. plumieri* and *E. gula* plus *G. cinereus* (Fig. 1), which correspond to two previously defined taxonomic assemblages characterized by the shape of the preoperculum; gerreids with a serrated preoperculum and gerreids with a smooth preoperculum, respectively. If currently recognized generic assignments are correct, the first assemblage would contain 11 species and the second 35 species. Although detailed systematic study of this family should be investigated with dense taxonomic sampling, this study provides (1) a baseline classification of the family, Gerreidae—two taxonomic sub-groups with distinguishable characters within the family should be valid and (2) useful molecular markers from both mitochondrial and nuclear genomes for future study on intrafamilial relationships of Gerreidae and other percoid families.

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