BIOCHEMICAL AND MORPHOMETRIC ANALYSES FOR PHYLOGENIC RELATIONSHIPS BETWEEN SEVEN SNAPPER SPECIES (SUBFAMILY LUTJANINAE) OF THE WESTERN ATLANTIC

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ABSTRACT

Fourteen snapper species belonging to the three genera (*Lutjanus* and two monotypic genera *Ocyurus* and *Rhomboplites*) of the subfamily Lutjaninae have been described in the western Atlantic. Electrophoretic (on 25 enzyme loci) and skull morphometric (by analysis of variance and discriminant analysis) comparisons among seven species of the three genera were performed. Average Nei's genetic distances were 0.566 ± 0.207 among five species of *Lutjanus*, 0.687 ± 0.148 between five species of *Lutjanus* and *O. chrysurus*, 0.869 ± 0.224 between five species of *Lutjanus* and *R. aurorubens*, and 0.877 between *O. chrysurus* and *R. aurorubens*. Cluster and additive tree analyses based on the genetic distance indicated that: 1) there are at least two distinct groups (gray and red snapper groups) within the genus *Lutjanus*; 2) the lane snapper (*L. synagris*) has a closer relationship with the red snapper group (*L. analis* and *L. vivanus*) than with the gray snapper group (*L. apodus* and *L. griseus*); and 3) there is a closer relationship between *Lutjanus* and *O. chrysurus* than between *R. aurorubens* and *Lutjanus* or *O. chrysurus*. On the other hand, the skull morphometric analysis indicated that: 1) the lane snapper (*L. synagris*) has greater affinity with the gray snapper group than with *L. analis* (red snapper); and 2) although *O. chrysurus* has some affinities with *R. aurorubens*, this monotypic genus showed much less distance than *R. aurorubens* from the *Lutjanus* group.

The subfamily Lutjaninae contains six genera. Three (*Lutjanus* and two monotypic genera *Ocyurus* and *Rhomboplites*) have representatives in the western Atlantic ocean, where fourteen species have been described (Allen, 1985; Anderson, 1986). These three genera are defined on the basis of 1) skull morphology, 2) presence or absence of pterygoid teeth, 3) basisphenoid shape, 4) number of gill rakers on lower limb, and 5) number of spinous dorsal finrays (Evermann and Marsh, 1900; Anderson, 1986). Evermann and Marsh (1900) suggested separation of *Rhomboplites aurorubens* from species of *Lutjanus* mainly because of the cranial differences. They also described numerous minor morphological peculiarities in *Ocyurus chrysurus*, where the skull morphology is also notably different from species of *Lutjanus*. These peculiarities have convinced some systematists to consider *Ocyurus* and *Rhomboplites* as monotypic genera. Based on the osteological observations, Vergara (1980) also described *O. chrysurus* and *R. aurorubens* to be highly apomorphic forms in the subfamily. Within the genus *Lutjanus*, on the other hand, Rivas (1966) proposed three groups based on external features such as meristic counts and coloration, in which *L. griseus*, *L. synagris* and *L. analis* are the representatives for each group (i.e., gray, lane, and red snappers, respectively). Vergara (1980) also proposed the same three groups according to osteological observations, suggesting that the *L. griseus* group is the least distant from the incipient form of this genus because of fewer apomorphic characters, and that the *L. griseus* and *L. mahogoni* (same as *L. synagris* group in Rivas) groups are plesiomorphic sister groups of the more apomorphic *L. vivanus* group (same as *L. analis* group in Rivas).

Thus, although considerable efforts for the systematics in this animal group have been performed (see Allen, 1985 for review), the phylogenetic relationships

between species of the different genera or between species within the genus *Lutjanus* are still unclear.

For biochemical systematic purposes, we have collected five species in the genus *Lutjanus* and two in the two monotypic genera. Parallel analyses of enzyme electrophoresis and skull morphometry were performed for these seven species. In this paper, we report the results and discuss the phylogenetic relationships among the three genera and the seven species.

Materials and Methods

Seven snapper species used in this study (Table 1) were mostly caught in Miami or Key West areas. A few of *Lutjanus synagris* and *Ocyurus chrysurus* were hatchery-raised specimens from parents captured in Miami.

Tissues (eye, heart, liver and muscle) were dissected and stored at −80°C until use. The tissues (0.2–0.3 g) were minced, homogenized in ice-cold distilled water (0.25 ml), and centrifuged at 13,000 × *g* for 10 to 20 sec at room temperature. Using the tissue extracts, horizontal starch gel electrophoresis was performed for 10 to 14 h at 75–100 volts at 4°C. Electrophoresis buffers and tissues for 15 enzyme systems used are listed in Table 2. The staining procedures were those described by Murphy et al. (1990). Using allele frequency data, Nei's genetic distance (D) (Nei, 1978) was calculated. Based on the D values, unweighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973) for cluster analysis and neighbor-joining method (NJM) (Saitou and Nei, 1987) for additive tree analysis were performed to infer phylogenetic relationships among species.

For morphometric analysis, 12 characters of the skull were measured to the nearest 0.1 mm (Fig. 1). Since the skulls of three out of four specimens of *L. vivanus* were damaged, this species was not included for morphometric comparison. Except for frontal (FW) and parietal (PW) widths, all characters have distinct apex, segment or projections to be measured. Transverse distance between dorsal apices of eye-orbits were measured as the frontal width (FW), and that between parietal-frontal sutures was adopted as parietal width (PW). Ratios of the eleven characters were calculated for the standard skull length (SSL) which was the distance from the tip of prevomer to ventral border between skull and atlas. Discriminant analysis (DIA) was performed using ratios of the eleven characters in order to calculate Mahalanobis distance between species. One-way analysis of variance (ANOVA) was applied to the arc-sign transformed ratios, and Tukey's test was performed for ranking species in each character.

Results

Electrophoresis.—Interpretations for enzyme-encoding presumptive gene loci were relatively simple in most enzyme systems except for calcium binding protein (Cbp) and malate dehydrogenase (Mdh). Although multiple bands or zones were observed in the gels stained for calcium binding protein, only the most anodal zone was scored reliably in comparison between species. Two or four banding patterns were observed in Mdh staining for muscle extract (Fig. 2). In all species, the most cathodal band was interpreted as a mitochondrial form of MDH polypeptide because of the apparent lack of affinity with the anodal polypeptides. In *L. analis* (La), *L. vivanus* (Lv) and *R. aurorubens* (Ra), it was assumed that the three most anodal bands consisted of two homodimers of *Mdh-1* and *Mdh-2*

Table 1. Seven snapper species used in this study

Subfamily	Genus	Species	Common name	Abbreviation
Lutjaninae	*Lutjanus*	*L. analis*	mutton	La
		L. apodus	school master	Lap
		L. griseus	gray	Lg
		L. synagris	lane	Ls
		L. vivanus	silk	Lv
	Ocyurus	*O. chrysurus*	yellow tail	Oc
	Rhomboplites	*R. aurorubens*	vermilion	Ra

Table 2. Enzymes and electrophoresis buffers used in this study

Enzyme	Locus	Tissue*	Buffer†
Acid phosphatase	*Acp*	L	TC6
Alcohol dehydrogenase	*Adh*	L	TVB
Aspartate aminotransferase	*Aat-1*	L	TC6
	Aat-2	L	
Calcium binding proteins	*Cbp*	M	TVB
Glycerol-3-phosphate dehydrogenase	*G3pdh-1*	L	TC6
	G3pdh-2	M	
Glucose-6-phosphate isomerase	*Gpi-1*	M, L	TC6
	Gpi-2	H	
L-Idotol dehydrogenase	*Iddh*	L	TC6
Isocitrate dehydrogenase	*Idh-1*	H, M	TC6
	Idh-2	L	
Lactate dehydrogenase	*Ldh-A*	E, H, M	TC6
	Ldh-B	E, H, L	
	Ldh-C	E	
Malate dehydrogenase	*Mdh-1*	H, M	TC6
	Mdh-2	H, M	
	mMdh	H, M	
Malic enzyme	*Me-1*	E, H, M	TC6
	Me-2	E, H, M	
Mannose-6-phosphate isomerase	*Mpi*	H, M	TC6
Phosphoglucomutase	*Pgm*	E, H, M	TC6
Phosphogluconate dehydrogenase	*Pgdh*	L, M	TC6
Pyruvate kinase	*Pk*	H, L	TC6
Superoxide dismutase	*Sod*	L	TC6

* E, eye; H, heart; L, liver; M, muscle.
† TC6 (Tris-citrate, pH 6.0) and TVB (Tris-Versene-Borate, pH 8.0) from Selander et al. (1971).

subunits and the heterodimer between them. In *L. vivanus*, a homodimer of *Mdh-2* subunits was assumed to have almost the same electrophoretic mobility with that of the mitochondrial form. On the other hand, in *L. apodus* (Lap), *L. griseus* (Lg), *L. synagris* (Ls), and *O. chrysurus* (Oc), only one anodal band was observed. We tentatively assumed that *Mdh-1* and *Mdh-2* subunits had almost the same electrophoretic mobility in these four species.

Aat-2, G3pdh-2, Idh-1, Ldh-A, Ldh-C and *Sod* were fixed for one allele for all species examined. Allele frequencies of the other nineteen loci are shown in Table 3. Nei's genetic distances (D) between species are calculated from data at all 25 loci (Table 4). The D values ranged from 0.178 to 0.898 with a mean of 0.566 $\pm$ 0.207 between five congeneric species of *Lutjanus*, from 0.585 to 0.975 with a mean of 0.687 $\pm$ 0.148 between five species of *Lutjanus* and *O. chrysurus*, from 0.603 to 1.177 with a mean of 0.869 $\pm$ 0.224 between five species of *Lutjanus* and *R. aurorubens*, and 0.877 between *O. chrysurus* and *R. aurorubens*. The intra- (between five species of *Lutjanus*) and inter- (between species of *Lutjanus* and *R. aurorubens*) generic averages of the genetic distances were significantly different (one-way ANOVA, $F = 6.245$, $P < 0.05$), suggesting that the electrophoretic comparison supports taxonomic separation between the two genera. On the other hand, the averaged genetic distance between species of *Lutjanus* and *O. chrysurus* was not different with that between five species of *Lutjanus* (one-way ANOVA, $F = 1.243$, $P > 0.1$). Cluster (UPGMA) and additive tree (NJM) analyses give similar impressions for the species relationships (Fig. 3 A, B). UPGMA suggests that there could be at least two groups within *Lutjanus*, where *L. apodus* (Lap) and *L. griseus* (Lg) (gray snapper group) are clustered separately from the other cluster containing *L. analis* (La) and *L. vivanus* (Lv) (red snapper group) and *L.*

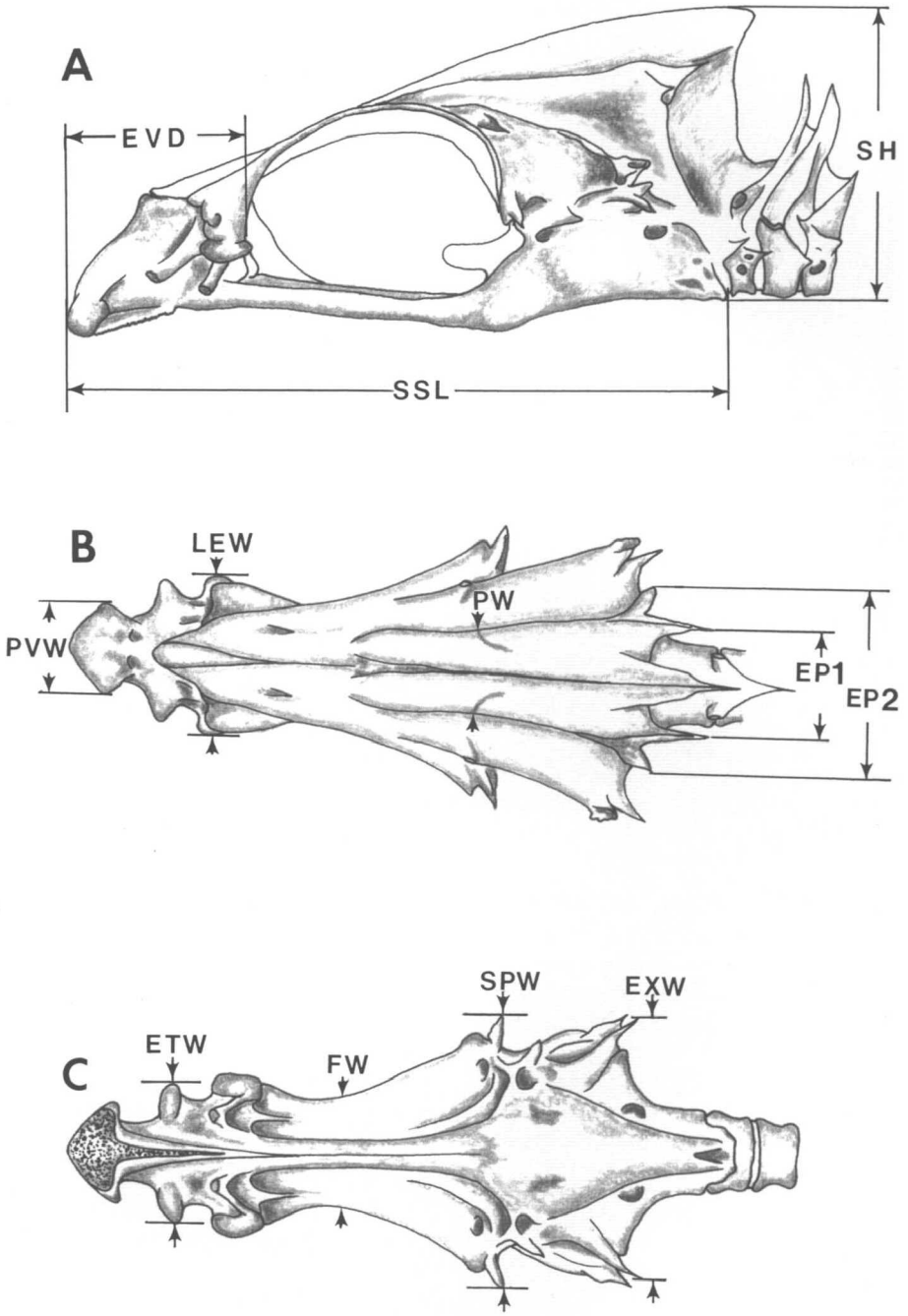

Figure 1. Lateral (A), dorsal (B) and ventral (C) views of the skull of *Lutjanus griseus* showing the 12 characters measured. Abbreviations are: (EP1) inner epiotic width, (EP2) outer epiotic width, (ETW) ethmoid width, (EVD) eye-vomer distance, (EXW) exoccipital width, (FW) frontal width, (LEW) lateral ethmoid width, (PVW) prevomer width, (PW) parietal width, (SH) skull height, (SPW) sphenotic width, and (SSL) standard skull length.

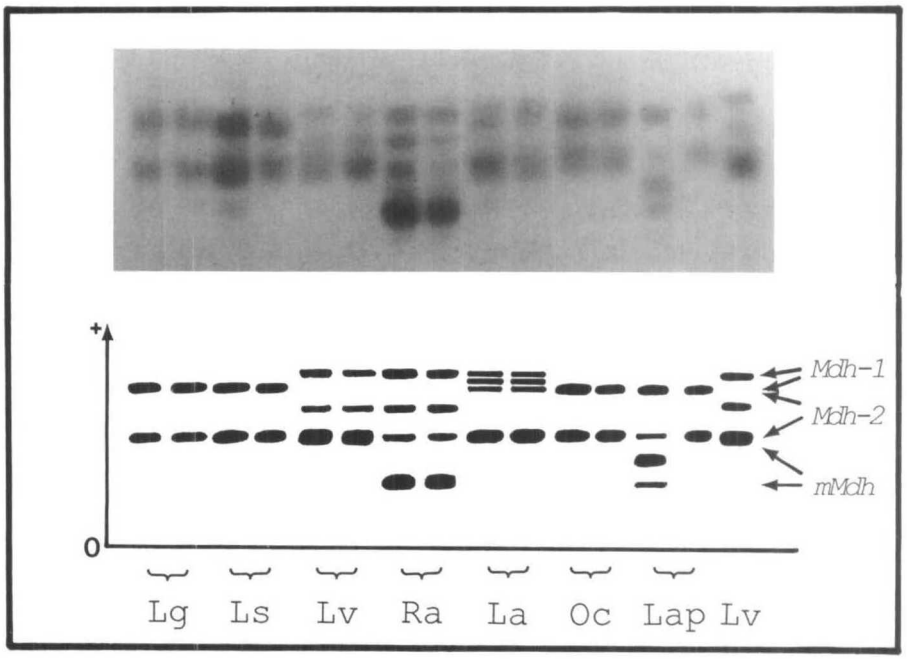

Figure 2. Electrophoretic phenotypes of malate dehydrogenase (MDH) in muscle of seven snapper species. In each species, the most cathodal band was interpreted as a mitochondrial form (*mMdh*). One of *L. apodus* (Lap) is heterozygote in *mMdh* locus. The three most anodal bands in *L. analis* (La), *L. vivanus* (Lv) and *R. aurorubens* (Ra) consist of two homodimers of *Mdh-1* and *Mdh-2* subunits and the heterodimer between them. Homodimer of *Mdh-2* subunits in *L. vivanus* shows the same mobility with that of *mMdh*. *Mdh-1* and *Mdh-2* homodimers and their heterodimers were assumed to have the same electrophoretic mobility in *L. apodus* (Lap), *L. griseus* (Lg), *L. synagris* (Ls) and *O. chrysurus* (Oc).

synagris (Ls). Similarly, NJM indicates that the gray snapper group (Lap and Lg) is located distantly from the red snapper group (La and Lv), and *L. synagris* (Ls) is located in between the two groups. On the other hand, the relationships among species of *Lutjanus* and the two outgroup species *O. chrysurus* and *R. aurorubens* may be better illustrated or understood by NJM than by UPGMA in which *O. chrysurus* keeps medium and similar distance from the gray snapper group (Lap and Lg) and from *L. analis* (La) and *L. synagris* (Ls), and *R. aurorubens* is more distant from the gray snapper group and *O. chrysurus,* but maintains medium distance from the red snapper group (La and Lv).

Morphometry.—Ratios (expressed as percent) of the 11 skull characters were plotted against standard skull length (SSL) (Fig. 4). Since a relatively wide and similar range of body sizes (juvenile through adult) was examined in *L. griseus* (Lg), *L. synagris* (Ls) and *O. chrysurus* (Oc), ratios which change through development (i.e., allometry) may be postulated. In all eleven characters, significantly positive or negative relationships between the ratios and standard skull length (SSL) were observed at least in one of the three species, and those species' plots are indicated with asterisk (*) in the figure. An increasing tendency was observed in ratios of the ethmoid width (ETW) and eye-vomer distance (EVD) of all three species, in those of the exoccipital (EXW), lateral ethmoid (LEW) and sphenotic (SPW) widths of *O. chrysurus,* in that of the frontal width (FW) of *L. griseus* and *O. chrysurus,* and in those of the prevomer width (PVW) and skull height (SH)

Table 3. Allele frequencies at 25 enzyme loci of seven snapper species

Species Loci	Alleles	La 7*	Lap 7	Lg 19	Ls 14	Lv 4	Oc 22	Ra 5
Acp	100	0.000	1.000	1.000	1.000	0.000	0.000	0.000
	60	1.000	0.000	0.000	0.000	1.000	1.000	1.000
Adh	150	0.000	0.000	0.000	0.094	0.000	0.000	0.000
	100	1.000	1.000	1.000	0.906	1.000	1.000	0.000
	90	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Aat-1	100	1.000	0.000	0.000	1.000	1.000	1.000	0.000
	90	0.000	0.000	0.000	0.000	0.000	0.000	1.000
	85	0.000	1.000	1.000	0.000	0.000	0.000	0.000
Cbp	140	0.000	1.000	1.000	0.000	1.000	0.000	0.000
	130	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	1.000	0.000	1.000	1.000
G3pdh-1	110	0.000	0.000	0.000	0.000	0.000	1.000	0.000
	100	0.000	1.000	1.000	1.000	0.000	0.000	0.000
	80	1.000	0.000	0.000	0.000	1.000	0.000	1.000
Gpi-1	100	1.000	1.000	1.000	1.000	0.000	0.167	0.000
	90	0.000	0.000	0.000	0.000	0.000	0.750	1.000
	80	0.000	0.000	0.000	0.000	1.000	0.083	0.000
Gpi-2	200	0.000	1.000	1.000	0.000	0.000	1.000	0.000
	100	1.000	0.000	0.000	1.000	1.000	0.000	1.000
Iddh	250	0.000	0.000	0.000	0.000	0.000	1.000	0.000
	180	0.000	1.000	1.000	0.000	0.000	0.000	0.000
	100	1.000	0.000	0.000	1.000	1.000	0.000	1.000
Idh-2	125	0.000	0.000	0.000	0.031	0.000	0.000	0.000
	100	1.000	0.000	0.000	0.969	1.000	0.000	0.833
	85	0.000	1.000	1.000	0.000	0.000	1.000	0.167
Ldh-B	100	0.000	1.000	0.000	1.000	1.000	0.000	1.000
	95	0.000	0.000	0.000	0.000	0.000	1.000	0.000
	85	0.000	0.000	1.000	0.000	0.000	0.000	0.000
	60	1.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-1	110	1.000	0.000	0.000	0.000	1.000	0.000	1.000
	100	0.000	1.000	1.000	1.000	0.000	1.000	0.000
Mdh-2	100	1.000	1.000	1.000	1.000	0.000	1.000	0.000
	60	0.000	0.000	0.000	0.000	1.000	0.000	1.000
mMdh	100	1.000	0.900	1.000	1.000	1.000	1.000	0.000
	70	0.000	0.100	0.000	0.000	0.000	0.000	1.000
Me-1	110	0.000	0.000	0.000	0.000	0.000	0.000	1.000
	100	1.000	1.000	0.000	1.000	1.000	0.000	0.000
	90	0.000	0.000	1.000	0.000	0.000	1.000	0.000
Me-2	105	0.000	0.000	0.000	0.000	0.000	0.000	1.000
	100	1.000	1.000	1.000	1.000	0.000	1.000	0.000
	95	0.000	0.000	0.000	0.000	1.000	0.000	0.000
Mpi	110	1.000	1.000	1.000	0.000	1.000	0.000	1.000
	100	0.000	0.000	0.000	1.000	0.000	1.000	0.000
Pgm	125	0.000	1.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	1.000	1.000	0.000	0.000
	95	0.000	0.000	1.000	0.000	0.000	0.000	0.000
	80	1.000	0.000	0.000	0.000	0.000	1.000	1.000
Pgdh	115	0.000	0.000	0.000	0.000	0.000	1.000	0.000
	100	1.000	0.000	0.000	1.000	1.000	0.000	0.000
	95	0.000	0.875	1.000	0.000	0.000	0.000	1.000
	85	0.000	0.125	0.000	0.000	0.000	0.000	0.000
Pk	100	0.000	1.000	1.000	1.000	1.000	0.000	0.000
	95	1.000	0.000	0.000	0.000	0.000	1.000	1.000

* Average number of individuals examined per locus. Loci fixed in one allele for all species are *Aat-2, G3pdh-2, Idh-1, Ldh-A, Ldh-C* and *Sod*.

Table 4. Genetic distances (Nei, 1978) among seven snapper species

	La	Lap	Lg	Ls	Lv	Oc	Ra
La							
Lap	0.725			0.566 ± 0.207			
Lg	0.782	0.178				0.687 ± 0.148	0.869 ± 0.224
Ls	0.441	0.438	0.543				
Lv	0.428	0.753	0.898	0.470			
Oc	0.606	0.679	0.584	0.590	0.975		
Ra	0.603	1.036	1.177	0.897	0.631	0.877	

of *L. synagris* and *O. chrysurus*. A decreasing tendency was observed in ratios of the inner and outer epiotic (EP1 and EP2) and sphenoid (SPW) widths of *L. synagris*, and in that of the parietal width (PW) of *L. griseus* and *L. synagris*. We assumed that the ratios showing positive or negative relationships with the standard skull length (SSL) would become constant after certain threshold(s). In fact, the plotted curves of most of the characters tend to be horizontal as size increases. Therefore, it is not correct to use all of the individual measurements. To lose as little data as possible, we defined the threshold at a standard skull length (SSL) of about 40 mm, although naturally the threshold, if any, might be different between species or characters. Specimens whose SSL was larger than this threshold were selected, and the ratios were used for ANOVA and DIA. Averages with the standard deviation of the ratios of eleven characters and the results of ANOVA with Tukey's test are shown in Table 5.

All ratios but one (ETW) showed heterogeneity among species. The cranial peculiarity of *R. aurorubens* (Ra) is quite evident, in which the ratios of EP1, EP2, EXW, FW, LEW, PW and SPW are predominantly larger, whereas those of EVD and PVW are smaller than in *Lutjanus*. This indicates that the skull of *R. aurorubens* is proportionally very wide compared with those seen in *Lutjanus*, except for the prevomer widths (PVW). The skull of *O. chrysurus* has proportional characters similar to those of *R. aurorubens*, in which ratios of frontal (FW) and parietal (PW) widths are larger, whereas that of prevomer width (PVW) is smaller, than in *Lutjanus*. All species of *Lutjanus* had similar skull morphometry, except that *L. analis* had larger ratios in eye-vomer distance (EVD) and skull height (SH)

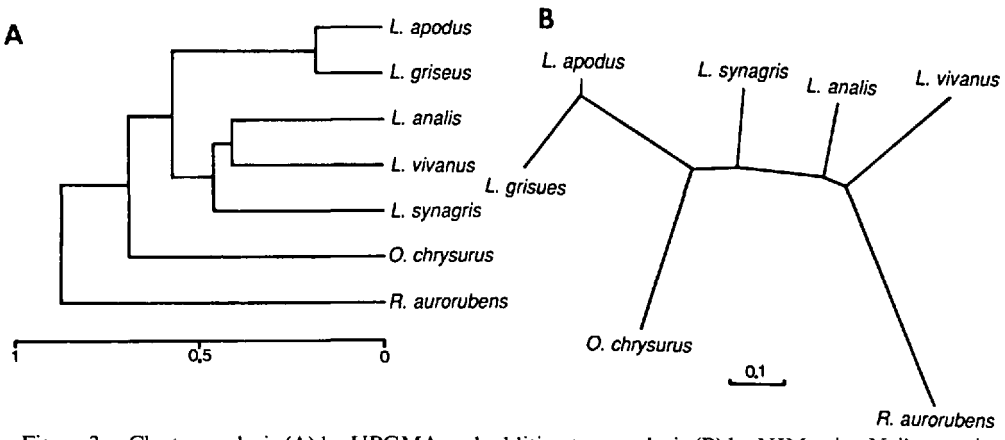

Figure 3. Cluster analysis (A) by UPGMA and additive tree analysis (B) by NJM using Nei's genetic distance values among seven snapper species. See Table 1 for abbreviations.

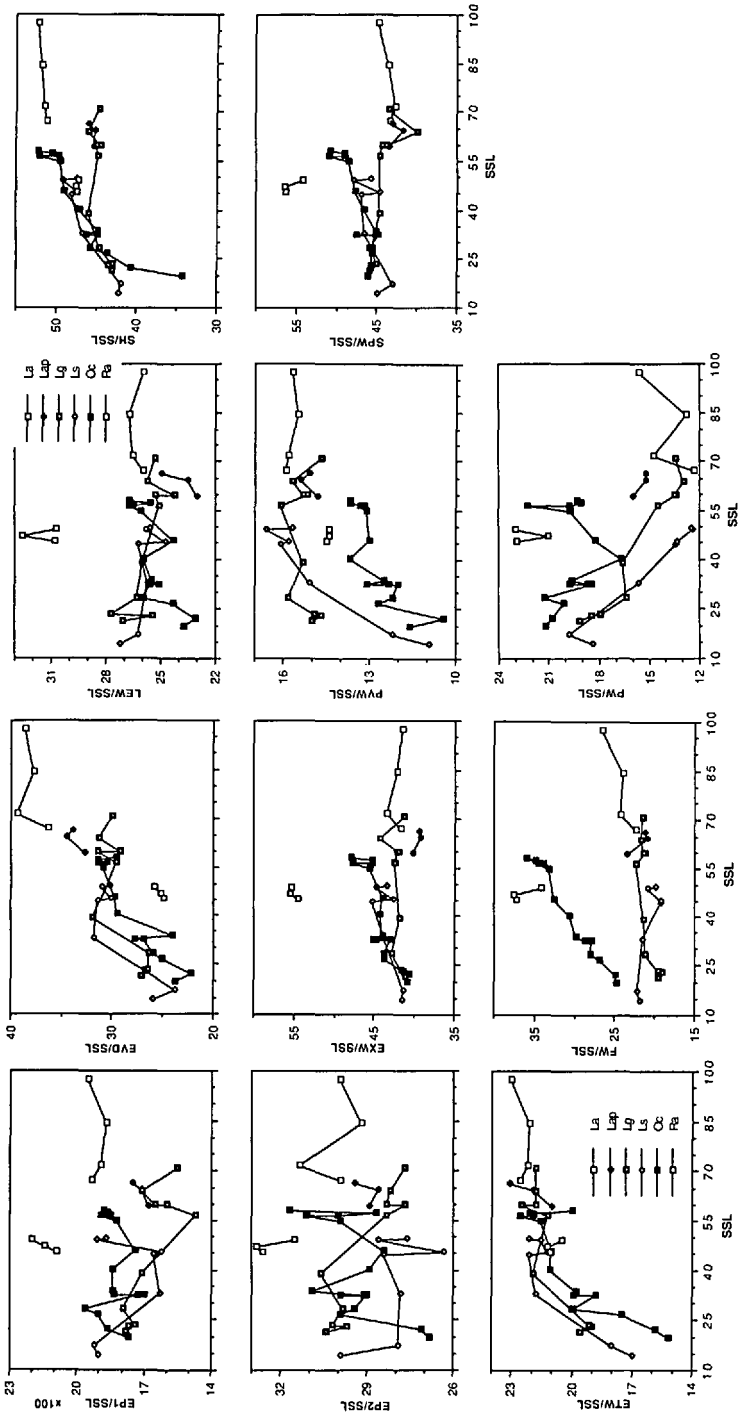

Figure 4. Ratios of 11 skull characters (expressed as percent on Y-axis) plotted against standard skull length (SSL) on X-axis. See Fig. 1 for abbreviations.

Table 5. Averages (±SD) and the range of the standard skull length (SSL) above hypothetical threshold (ca 40 mm) and averages of ratios (±SD) of 11 characters in six snapper species

Species Characters	La 4*	Lap 3	Lg 6
SSL† (mm)	80.2 ± 11.8	63.4 ± 2.9	58.4 ± 9.7
(range)	(67.3–97.4)	(59.5–66.3)	(39.2–70.8)
PE1	0.191 ± 0.003^{ab}§	0.171 ± 0.003^{bc}	0.162 ± 0.009^{c}
EP2	0.301 ± 0.008^{ab}	0.290 ± 0.003^{ab}	0.287 ± 0.010^{b}
ETW	0.225 ± 0.004^{a}	0.220 ± 0.009^{a}	0.218 ± 0.004^{a}
EVD	0.380 ± 0.012^{a}	0.336 ± 0.007^{b}	0.305 ± 0.010^{bc}
EXW	0.422 ± 0.007^{bc}	0.396 ± 0.004^{c}	0.419 ± 0.003^{bc}
FW	0.243 ± 0.015^{b}	0.218 ± 0.012^{b}	0.217 ± 0.004^{b}
LEW	0.263 ± 0.004^{b}	0.238 ± 0.008^{b}	0.253 ± 0.006^{b}
PVW	0.157 ± 0.010^{a}	0.151 ± 0.002^{ab}	0.154 ± 0.004^{ab}
PW	0.139 ± 0.014^{c}	0.155 ± 0.004^{bc}	0.142 ± 0.012^{c}
SH	0.517 ± 0.004^{a}	0.454 ± 0.004^{c}	0.451 ± 0.006^{c}
SPW	0.435 ± 0.008^{c}	0.427 ± 0.007^{c}	0.441 ± 0.012^{c}

* Number of individuals examined.
† See Figure 2 for the abbreviations.
§ Averages carrying different superscripts are significantly different from one another.

than the other species. Differences between genera may be roughly estimated by comparing the counts of significantly different characters (see Table 6, above diagonal), where those between congeneric species within *Lutjanus* ranged from 0 to 3, those between species of *Lutjanus* and *O. chrysurus* ranged from 3 to 6, and those between species of *Lutjanus* and *R. aurorubens* ranged from 5 to 9. Although Mahalanobis distances (Table 6, below diagonal) were significantly correlated with the counts of significantly different characters ($r = 0.838$, $P < 0.005$), a few discrepancies between these values are observed. For example, *O. chrysurus* shows moderate counts of significantly different characters from *Lutjanus*, but the Mahalanobis distance are comparable with those of *L. analis*. This indicates that *Ocyurus* has a number of significantly but slightly different characters from species of *Lutjanus*, while *L. analis* has fewer significantly and eminently different characters from its congeneric members. Thus, Mahalanobis distance may be more appropriate to illustrate morphometric difference among species along with the genetic differentiation. Cluster analysis (UPGMA) of Mahalanobis distances (Fig. 5) indicates that *L. synagris* (Ls) is morphometrically more similar to gray snappers (Lap and Lg) than to red snappers (La) and that, unlike *R. aurorubens*, *O. chrysurus* is not clustered out of *Lutjanus*.

Discussion

The external characters by which Rivas (1966) strictly separated three groups in the genus *Lutjanus* are listed in Table 7. Lane and red snapper groups share

Table 6. Number of characters which are significantly different (by Tukey's test) (above diagonal) and Mahalanobis distance (by DIA) (below diagonal) between species

Species	La	Lap	Lg	Ls	Oc	Ra
La	–	2	3	2	5	8
Lap	13.0	–	0	1	6	7
Lg	17.0	7.3	–	0	6	8
Ls	18.8	12.2	8.8	–	3	9
Oc	21.7	16.6	14.2	18.1	–	5
Ra	47.9	40.1	35.0	35.7	29.0	–

Table 5. Extended

Ls 4	Oc 7	Ra 3	*F*	*P*
47.4 ± 2.2	52.9 ± 6.4	47.3 ± 1.6		
(44.7–49.7)	(40.2–58.4)	(45.4–49.2)		
0.177 ± 0.013^{bc}	0.184 ± 0.005^{b}	0.214 ± 0.004^{a}	19.92	<0.001
0.277 ± 0.009^{b}	0.298 ± 0.012^{ab}	0.323 ± 0.006^{a}	8.30	<0.001
0.217 ± 0.004^{a}	0.215 ± 0.008^{a}	0.209 ± 0.003^{a}	2.34	>0.05
0.306 ± 0.005^{bc}	0.304 ± 0.007^{c}	0.253 ± 0.004^{d}	68.52	<0.001
0.441 ± 0.010^{b}	0.457 ± 0.014^{b}	0.551 ± 0.005^{a}	93.69	<0.001
0.198 ± 0.007^{b}	0.336 ± 0.016^{a}	0.363 ± 0.015^{a}	104.70	<0.001
0.256 ± 0.005^{b}	0.260 ± 0.008^{b}	0.314 ± 0.009^{a}	36.5	<0.001
0.161 ± 0.004^{a}	0.134 ± 0.003^{c}	0.144 ± 0.001^{bc}	44.69	<0.001
0.129 ± 0.005^{c}	0.193 ± 0.015^{ab}	0.223 ± 0.009^{a}	30.58	<0.001
0.480 ± 0.007^{bc}	0.500 ± 0.017^{ab}	0.474 ± 0.001^{bc}	25.19	<0.001
0.463 ± 0.012^{bc}	0.489 ± 0.014^{b}	0.556 ± 0.010^{a}	58.83	<0.001

six out of the seven characters, coinciding with cluster analysis based on the genetic distance in the present study. Conversely, our skull morphometric analysis supports Vergara (1980) who categorized gray and lane snappers as a sister group of red snapper. This discrepancy might be rectified by assuming the lane snapper group to be more of an intermediate form than Vergara (1980) expected. For example, Rivas (1966) included *L. buccanella* (blackfin snapper) in the red snapper group, whereas Vergara (1980) placed this species in the lane (or mahogony) snapper group. As shown by an additive tree analysis, lane snapper (Ls) is indeed situated in a position intermediate between the gray and red snapper groups. Therefore, rather than three distinct groups in this genus of the western Atlantic snappers, we suggest that there are two well-defined groups (gray and red) as already suggested by Rivas (1966) and Vergara (1980) and one genetically and morphologically plastic group (lane snapper group) which possesses affinity for the two distinct groups.

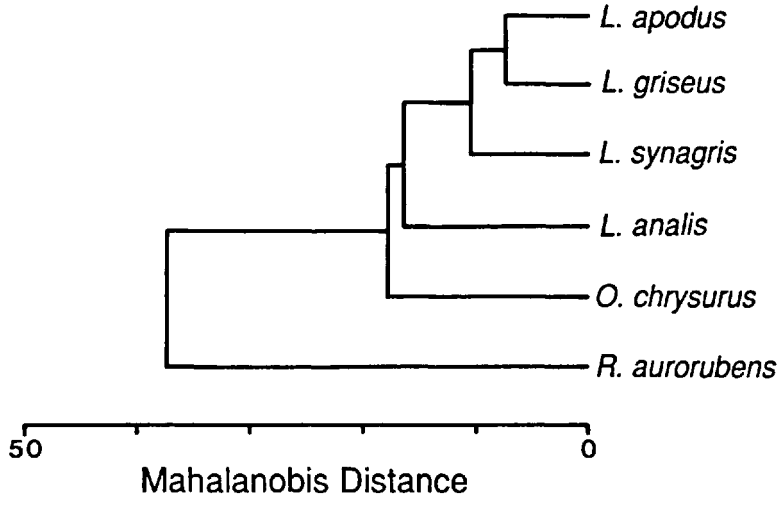

Figure 5. UPGMA cluster analysis using Mahalanobis distances based on 11 skull morphometric characters among six snapper species.

Table 7. Characters Rivas (1966) used for grouping *Lutjanus* species of the Western Atlantic

Character	Group		
	Gray	Lane	Red
Scales above opercle	2–3 rows	4–7	4–7
Lateral scales	40–48(41–47)	46–53(47–51)	46–53(47–51)
Jaws	subequal or upper is longer	lower is longer	lower is longer
Accessory lateral lines on caudal fin	usually absent	present	present
Dorsal rays	14	12	14
Lateral spot	absent	present	present or absent
Color	not red	usually red	usually red
Species	*L. apodus* *L. griseus* *L. cyanopterus* *L. jocu*	*L. mahogoni* *L. synagris*	*L. analis* *L. buccanella* *L. campechanus* *L. purpureus* *L. vivan*

There has been little doubt that *Rhomboplites* should stand as a monotypic genus, and this is supported by both our electrophoretic and morphometric analyses. Within the three genera, *Rhomboplites* may be the earliest offshoot as already suggested by Johnson (1981). As is well known, *R. aurorubens* and *O. chrysurus* share some characters such as pterygoid teeth, high numbers of gill rakers, a deeply forked caudal fin, and small canines. Furthermore, in the present study we observed proportionally wider frontal and parietal bones and narrower prevomer of these two species than in *Lutjanus*. Therefore, one might be tempted to consider *R. aurorubens* and *O. chrysurus* as sister groups distinct from *Lutjanus*. However, our biochemical and morphometric analyses indicate a closer relationship between species of *Lutjanus* and *O. chrysurus* and could not even rule out inclusion of *O. chrysurus* into the genus *Lutjanus*. Therefore, the similar characters shared between *O. chrysurus* and *R. aurorubens* could be convergence for ecological adaptation. Thus, the status of *Ocyurus* as a monotypic genus is open to challenge. To test this hypothesis, biochemical comparisons between Atlantic and Pacific snappers would be useful, because separation of the two genera *Lutjanus* and *Ocyurus* must have occurred before closing of the Isthmus of Panama.

Acknowledgments

We thank C. R. Robins, University of Miami, for his kind reading and valuable comments on the manuscript and K. A. Kilpatrick for helping us to use the SYSTAT program. M. E. Clarke is also gratefully acknowledged for providing us valuable snapper samples. This research was supported by NOAA/CIMAS contract NA90-RA-H-00075.

Literature Cited

Allen, G. R. 1985. FAO species catalogue. Snapper of the world. An annotated and illustrated catalogue of lutjanid species known to date. FAO Fish. Synop. No. 125, Vol. 6. 208 pp.

Anderson, W. D., Jr. 1986. Review of the systematics of the fishes of the family Lutjanidae (Perciformes: Percoidei), the snappers. Pages 1–31 *in* S. Ralston and J. J. Polovina, eds. Biology and fisheries management of tropical groupers and snappers. Westview Press, Boulder, Colorado.

Evermann, B. W. and M. C. Marsh. 1900. The fishes of Porto Rico. Bull. U.S. Fish. Comm. (1899): 51–350.

Johnson, G. D. 1981. The limits and relationships of the Lutjanidae and associated families. Bull. Scripps Inst. Oceanogr. 24: 1–114.

Murphy, R. W., J. W. Sites, Jr., D. G. Buth and C. H. Haufler. 1990. Proteins I: isozyme electro-

phoresis. Pages 45–126 *in* D. M. Hillis and C. Moritz, eds. Molecular systematics. Sinauer Assoc, Massachusetts.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Rivas, L. R. 1966. Review of the *Lutjanus campechanus* complex of red snappers. Q. J. Fl. Acad. Sci. 29: 117–136.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Selander, R. K., S. Y. Smith, S. Y. Yang, W. E. Johnson and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud. Genet. VI. Univ. Texas Publ. 7103: 49–90.

Sneath, P. H. and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman, San Francisco. 573 pp.

Vergara, R. R. 1980. Phylogenetic considerations on the Cuban species of the genus *Lutjanus* (Lutjanidae, Perciformes, Teleostei). Acad. Cienc. Cuba, no. 113, 39 pp. (In Spanish with English abstract)

DATE ACCEPTED: June 3, 1991.

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