

Molecular Phylogenetics and Ecological Diversification of the Transisthmian Fish Genus *Centropomus* (Perciformes: Centropomidae)

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Phylogenetic relationships among the 12 recognized fish species in the New World genus *Centropomus* (Pisces, Centropomidae) were analyzed using allozyme electrophoresis and 618 bp of the mitochondrial DNA 16S ribosomal RNA (rRNA) gene. Molecular phylogenetic trees were generally consistent with previously published partial hypotheses based on morphological evidence. However, previously undefined sister group relationships between major species groups were resolved using molecular data, and phylogenetic hypotheses for *Centropomus* based on 16S rRNA sequences were better supported than were allozyme-based hypotheses. The high level of congruence among the trees inferred from the nuclear and mitochondrial characters provided a firm phylogenetic basis for analysis of ecological diversification and molecular evolution in the genus. Compared to basal *Centropomus* species, members of the most nested species group were significantly larger in body size and occupied a marine niche only peripherally utilized by their congeners. We also observed substitution rate heterogeneity among 16S rRNA lineages; in contrast to expectations based on “metabolic rate” and “generation interval” models, relative substitution rates were faster than expected for the group of large-bodied snooks. Using the Pliocene rise of the Central American isthmian marine barrier to calibrate rates of 16S ribosomal gene evolution in *Centropomus*, we found that the rates for the genus were similar to those reported for higher vertebrates. Analysis of the three sets of transisthmian geminate taxa in *Centropomus* indicated that two of the pairs were probably formed during the Pliocene rise of the isthmus while the third pair diverged several million years earlier. © 1999 Academic Press

Key Words: molecular phylogenetics; allozymes; 16S ribosomal RNA; Centropomidae; rate heterogeneity; transisthmian pairs; divergence times; ecological diversification

INTRODUCTION

The Centropominae, a New World subfamily of tropical and subtropical fishes, is represented by a single genus, *Centropomus* Lacépède, 1802. Commonly known as snooks or robalos, centropomines are important high-level carnivores in eastern Pacific and western Atlantic coastal waters and associated estuaries and rivers. Six species are known from the eastern Pacific; they range between northern Mexico (Gulf of California) and Peru. Six species have also been identified in the western Atlantic; they range between the Florida peninsula and Brazil, including islands of the Greater and Lesser Antilles. No species occurs in both Atlantic and Pacific waters. However, most snook species are broadly distributed latitudinally within the general range of the genus (Rivas, 1986). *C. poeyi* has the most restricted distribution and occurs only along the Mexican coast in the Bay of Campeche region of the Gulf of Mexico (Chávez, 1961).

Centropomines belong to the perch family Centropomidae (Poey, 1868), a diverse assemblage of circum-tropical, basal percoid fishes (Gosline, 1966). Fraser (1968), Greenwood (1976), and Rivas (1986) have hypothesized phylogenetic relationships within *Centropomus* based on morphology (Fig. 1); however, their hypotheses were largely untested by critical or statistical analyses. Only Greenwood (1976) attempted to define apomorphic and plesiomorphic character states for snook but the mosaic nature of morphological character evolution precluded a detailed phylogenetic hypothesis. Both Fraser (1968) and Rivas (1986) agreed that at least three major species groups exist within *Centropomus* but disagreed on certain sister group relationships within those groups (Figs. 1A and 1C). Rivas (1986) named each major group by a representative species (e.g., the *C. undecimalis* species group). Greenwood (1976) accepted two of the three putative groups as valid but grouped *C. pectinatus* (its sibling

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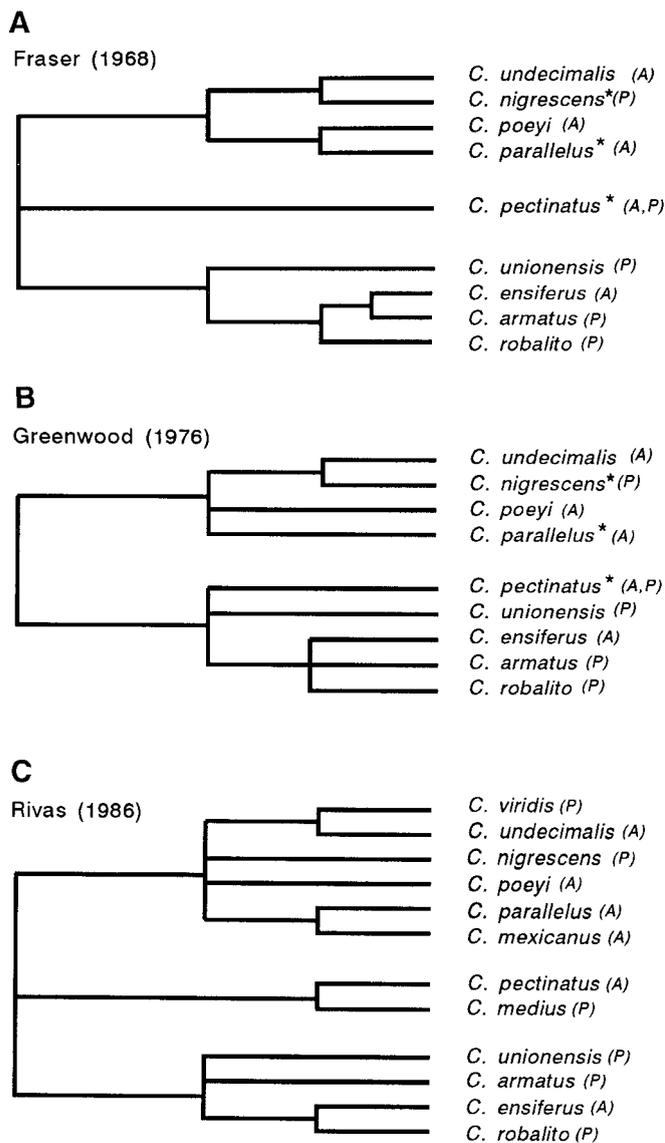


FIG. 1. Published phylogenetic relationships of members of Centropominae. Asterisks (*) denote taxa composed of species pairs not recognized prior to Rivas' (1986) revision of the genus. Letters in parentheses denote occurrence of species in coastal waters of the following oceans: A, Atlantic; P, Pacific. (A) Relationships proposed by Fraser (1968) based on an examination of skull osteology. Fraser did not examine material from *C. nigrescens*; his placement of this species was based on his interpretation of data reported by Chávez (1961). (B) Phylogeny according to the osteological and morphological analyses of Greenwood (1976). (C) Phylogeny according to the morphological analysis of Rivas (1986). In Rivas' revision of the genus, three new species (*C. viridis*, *C. mexicanus*, and *C. medius*) were recognized and described.

species *C. medius* was not recognized at the time) within the *C. ensiferus* group rather than affording it a phylogenetic status equal to the other groups (Fig. 1B). Phylogenetic relationships between major species groups within *Centropomus* are unresolved.

Because of the constituent species' morphological

similarities, the genus *Centropomus* has been described as a "compact, homogeneous group" highly distinct from its two centropomid confamilials, *Lates* and *Psammoperca* (subfamily Latinae [Jordon, 1923]). However, a notable example of morphological variation within *Centropomus* is the large range of maximum body sizes found in the various snook species (Table 1). Morphological diversification often occurs in conjunction with ecological shifts (Richman, 1996), and as a group, snook are ecologically diverse, particularly in the habitats occupied by adults. Thus, an examination of body sizes and habitat preferences among snook species within the comparative framework provided by an independent, molecular-based phylogeny (Felsenstein, 1985a) may offer *prima facie* insight into the direction of the biological and ecological change in the Centropominae over evolutionary time.

For *Centropomus*, a molecular phylogenetic hypothesis can also provide the framework required to investigate evolutionary rates (Donaldson and Wilson, 1997) and times of phylogenetic separation of species groups within the genus. The Pliocene emergence of the Central American isthmus 2.9–3.5 mya (Keigwin, 1982; Coates *et al.*, 1992) provides a convenient exogenous time marker for studies of molecular divergence and speciation for other neotropical marine and freshwater taxa (Bermingham *et al.*, 1997 and references therein; Bermingham and Martin, 1998). This geologic event putatively produced several transisthmian geminate species in *Centropomus* (Fraser, 1968; Rivas, 1986). Establishing centropomine sister group and outgroup relationships allows Zuckerkandl and Pauling's (1965) molecular clock hypothesis to be used to examine relative rates of evolution among geminate taxa. Divergence rates can then be estimated from transisthmian sister taxa under the assumption of allopatric separation initiated by the rise of the Central American isthmus (e.g., Bermingham *et al.*, 1997; Donaldson and Wilson, 1997).

Here, we generate a robust molecular phylogeny inferred from the analyses of allozymes and mtDNA 16S ribosomal RNA (rRNA) for *Centropomus*. Using the molecular hypothesis as a phylogenetic framework, we present a comparative analysis of the evolution of certain morphological and ecological characters within the genus. We also examine rate variation in mtDNA 16S rRNA lineages across centropomine taxa. Finally, utilizing the vicariant history of the geminate centropomine species, we examine the evolutionary rate of mtDNA 16S rRNA genes within the genus and estimate divergence times for multiple sets of transisthmian taxa.

MATERIALS AND METHODS

Sample Collection

The species utilized and their locations of collection, sample sizes, distributions, and life history components

are listed in Table 1. Field collections were made by hook and line fishing, netting, or electro-shocking. The *C. undecimalis* sample from Trinidad and several samples of the Pacific species were obtained from fish markets. Tissue samples of snook collected from western Atlantic coastal and riverine waters were frozen using liquid nitrogen or dry ice. Samples of the two outgroup species *Lates calcarifer* and *L. niloticus* were provided by Dr. Clive Keenan (University of Queensland, Australia). The tissues were stored at -80°C in our laboratory until processing. Specimens of the genus *Centropomus* were identified using the taxonomic key

of Rivas (1986). Elsewhere, we maintain that results of intra- and interspecific genetic analyses are consistent with Rivas' (1986) taxonomic revision of *Centropomus* (Tringali *et al.*, in press).

Allozyme Electrophoresis

Allozyme electrophoretic procedures followed Tringali and Bert (1996). Nineteen histochemical stains were used to resolve a total of 27 putative gene loci for all *Centropomus* and outgroup taxa (Table 2). For each species, BIOSYS-1 (Swofford and Selander, 1981) was used to generate allele frequencies. We examined phylo-

TABLE 1
The Subfamily Centropominae and the Outgroups Used in This Study

	Collection location and sample size ^a	Distribution ^b	Adult habitat ^b	Maximum size ^c (mm TL/kg TW)
Order Perciformes				
Family Centropomidae				
Subfamily Latinae				
<i>Lates niloticus</i>	Lake Victoria, Kenya (17/2)	Northwest Africa	Rivers and lakes	2000/87.0
<i>L. calcarifer</i>	Burdekin River, Queensland, Australia (12/0)	Indo-Pacific	Marine and estuarine	1520/28.7
Subfamily Centropo- minae				
<i>Centropomus undecimalis</i>	Nine western Atlantic locations ^d (259/2)	Tropical western Atlantic	Marine, estuarine, and riverine	1395/35.0
<i>C. viridis</i>	Panama (4/2)	Tropical eastern Pacific	Marine and estuarine	1100/23.8
<i>C. poeyi</i>	Laguna de Alvarado, Alvarado, Mexico (10/2)	Southwestern Gulf of Mexico	Marine, estuarine, and riverine	1035/15.0
<i>C. nigrescens</i>	Aguadulce and other locations, Panama (3/3)	Tropical eastern Pacific	Marine and estuarine	1171/26.2
<i>C. parallelus</i>	Loxahatchee River, Florida, USA (15/1) Sebastian River, Florida, USA (16/1) Rio Colorado, Costa Rica (2/1) Rio Anasco and LaBomba, Puerto Rico (13/0)	Tropical western Atlantic	Estuarine and riverine	630/3.8
<i>C. mexicanus</i>	Rio Anasco and LaBomba, Puerto Rico (9/2)	Tropical western Atlantic	Estuarine and riverine	430/nd
<i>C. pectinatus</i>	Loxahatchee River, Florida, USA (9/1) Rio Colorado, Costa Rica (6/1)	Tropical western Atlantic	Estuarine and riverine	608/1.5
<i>C. medius</i>	Aguadulce and other locations, Panama (4/2)	Tropical eastern Pacific	Estuarine and riverine	558/1.3
<i>C. ensiferus</i>	Loxahatchee River, Florida, USA (4/0) Rio Colorado, Costa Rica (3/0) Rio Anasco, Puerto Rico (9/2)	Tropical western Atlantic	Riverine	390/0.6
<i>C. unionensis</i>	Panama (6/2)	Tropical eastern Pacific	Riverine	366/nd
<i>C. armatus</i>	Panama (4/3)	Tropical eastern Pacific	Riverine	370/nd
<i>C. robalito</i>	Aguadulce, Panama (5/2)	Tropical eastern Pacific	Riverine	345/nd

^a Numbers in parentheses indicate the number of individuals from each location assayed for allozymes and 16S rRNA, respectively.

^b For members of the genus *Lates*, distribution and habitat data was obtained from Greenwood (1976); for members of *Centropomus*, the data was obtained from Rivas (1986).

^c Maximum reported sizes for the *Lates* species and for *C. viridis* and *C. medius* were obtained from International Game Fish Association (IGFA) records; maximum sizes for the remaining *Centropomus* species were obtained from Rivas (1986), who conducted a literature survey that included IGFA records; maximum sizes for *C. pectinatus* and *C. ensiferus* were obtained from pending IGFA world records (R. Taylor, pers. comm.). mm TL, total length in mm; kg TW, total weight in kg; nd, not determined.

^d Sample locations and additional allozyme results appear in Tringali and Bert (1996). Sample locations for the individuals sequenced for 16S rRNA were Port-O-Spain, Trinidad, and Jupiter Inlet, Florida, USA.

TABLE 2
Enzymes and Staining Procedures Used in Allozyme Electrophoresis of Centropomidae

Protein ^a	Enzyme number	Number of loci	Buffer system ^b	Tissue used ^c	Enzyme structure ^d
Aspartate aminotransferase (<i>AAT</i>)	2.6.1.1	4	5	s, l	d
Acid phosphatase (<i>ACP</i>)	3.1.3.2	1	5	l	d
Aldehyde oxidase (<i>AO</i>)	1.2.3.1	1	2	s, l	?
Esterase (<i>EST</i>)	3.1.1.-	2	2	l	m
General protein (<i>GP</i>)	—	3	2	s	m
alpha-Glycerophosphate dehydrogenase (<i>αGPD</i>)	1.1.1.8	1	9	s, l	d
Glucose phosphate isomerase (<i>GPI</i>)	5.3.1.9	2	3	s	d
Hexanol dehydrogenase (<i>HDH</i>)	1.1.1.71	1	3	s, l	?
Isocitrate dehydrogenase (<i>IDH</i>)	1.1.1.42	1	9	s, l	d
L-Lactate dehydrogenase (<i>LDH</i>)	1.1.1.27	2	3	s, l	t
Malate dehydrogenase (<i>MDH</i>)	1.1.1.37	1	5	l	?
Malic enzyme (<i>ME</i>)	1.1.1.40	1	6	l	?
Mannose-6-phosphate isomerase (<i>MPI</i>)	5.3.1.8	1	2	l	m
Dipeptidase (<i>PEPA</i>) (specific for phenylalanyl-leucine)	3.4.-.	1	8	l	d
Tripeptidase (<i>PEPB</i>) (specific for leucyl-glycyl-glycine)	3.4.-.	1	9	l	d
6-Phosphogluconate dehydrogenase (<i>6PGD</i>)	1.1.1.44	1	5	l	?
Phosphoglucomutase (<i>PGM</i>)	5.4.2.2	1	8	l	m
Superoxide dismutase (<i>SOD</i>) ^e	1.15.1.1	1	3	s, l	?
Xanthine dehydrogenase (<i>XDH</i>)	1.1.1.204	1	6	l	d

Note. For locus nomenclature, we followed the recommendations of Shaklee *et al.* (1992), except that we omitted the asterisk from the locus abbreviation.

^a Proteins were resolved by the electrophoretic procedures of Selander *et al.* (1971).

^b All buffer system recipes may be found in Selander *et al.* 1971.

^c s, somatic muscle; l, liver.

^d m, monomer (presumed heterozygotes show two bands); d, dimer (presumed heterozygotes show three bands); t, tetramer (presumed heterozygotes show five bands).

^e Resolved on HDH and ME stains.

genetic relationships between taxa employing both maximum-parsimony (MP) and phenetic analyses. For the MP analysis, the branch-and-bound search, majority consensus, and bootstrap options in PAUP (Version 3.1; Swofford, 1993) were used to generate maximum-parsimony trees from a data matrix in which loci were considered to be unordered, multistate characters (Sole-Cava *et al.*, 1994) and in which allelic states were quantitatively coded (Buth, 1984). Bootstrap confidence levels (Felsenstein, 1985b) were estimated using 500 replications. For the phenetic analysis, Nei's D was computed for all pairwise combinations of taxa over all loci using BIOSYS-1. These distances were used to reconstruct neighbor-joining (NJ) and UPGMA trees using the programs NJTREE and UPGMA, respectively (Jin and Ferguson, 1990); standard errors for branching points of the UPGMA tree were computed as in Nei *et al.* (1985).

Sequencing and Analysis of mtDNA 16S rRNA

Total genomic DNA was extracted from white muscle or liver tissue by proteinase K/SDS lysis and purified by phenol:chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989). The polymerase chain reaction (PCR, Saiki *et al.*, 1988) was used to amplify one segment of approximately 618 bp from the mtDNA 16S rRNA gene. PCR primer sequences for the 16S rRNA

gene were obtained from Palumbi *et al.* (1991). The positions of the 3' end of each primer in the human mitochondrial genome are 2510 and 3080, respectively (Anderson *et al.*, 1981). Double-stranded PCR amplification was performed in a 25- μ l aqueous solution containing 2.5 μ l of 8 mM dNTP, 2.5 μ l of 10 \times Taq buffer (Kocher *et al.*, 1989), 2.5 μ l of each primer (10 μ M), 1 μ l of genomic DNA (approximately 0.1–1.0 μ g), and 0.1 μ l of Amplitaq DNA polymerase (Perkin-Elmer-Cetus). Following an initial denaturation step for 1 min at 94°C, amplification was carried out over 28 cycles, using the following temperature profile: denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min with a 1-s increase in extension time per cycle. Negative controls containing all reagents except template DNA were included in all amplifications.

Unmodified PCR products were directly cloned into a T-vector (Bluescript PBC KS-, Stratagene) using the procedure of Marchuk *et al.* (1990). Positive recombinants were identified using restriction enzyme analysis of plasmid preparations for which the DNA was digested with PvuII (Klein *et al.*, 1980). Plasmid DNA was prepared from 3-ml cultures of positive recombinant cells incubated overnight at 37°C (Sambrook *et al.*, 1989); purified DNA templates were denatured

according to the protocol of Hattori and Sakaki (1986). Dideoxy sequencing (Sanger *et al.*, 1977) was performed in both directions using T3 and T7 primers (Stratagene), Sequenase (Version 2.0, U.S. Biochemicals), and [S^{35}]dATP (Dupont Biotechnology Systems), following the U.S. Biochemicals protocol. The products of the sequencing reactions were electrophoresed in 6% polyacrylamide gels containing 7 M urea, fixed using 7% acetic acid and 10% methanol, vacuum dried, and autoradiographed.

Sequences for all taxa were aligned using the ESEE multiple sequence editor (Cabot and Beckenbach, 1989). Using MEGA (Version 1.01; Kumar *et al.*, 1993), we calculated base compositional frequencies and pairwise transition/transversion (TS/TV) ratios for all taxa. The potential for saturation of transitions was evaluated by comparing TS/TV ratios against uncorrected percentage divergences for each pair of taxa.

We examined the phylogenetic relationships between species based on the rRNA sequence using MP, minimum-evolution (ME [Rzhetsky and Nei, 1992]), and maximum-likelihood (ML [Felsenstein, 1981]) analyses. The MP analysis was performed using PAUP. Indels were alternatively treated as a fifth character state or as missing data in this analysis. Parsimony analyses were done considering TSs and TVs equally and using an *a priori* weighting scheme (Swofford *et al.*, 1996) in which a TS/TV weighting of 1:2 was employed. To assess the statistical significance of phylogenetic groupings generated using the branch-and-bound algorithm, we used a bootstrap analysis; bootstrap confidence intervals were estimated using 500 replications, and a bootstrap consensus tree was reconstructed. We examined the phylogenetic content of our data by checking the g_1 statistic for the distribution of 10,000 randomly generated trees (Hillis and Huelsenbeck, 1992). This procedure was repeated employing trees constrained to well-defined clades. Estimates of the shape parameter for the gamma model of among-site rate variation were made using the GAMMA program (Sullivan *et al.*, 1995). NJ and ME trees were generated using pairwise distances obtained from the gamma-corrected Kimura's (1980) two-parameter model (MEGA). For each pair of sequences in which one or both taxa contained an indel at a nucleotide position, those sites were excluded. We searched for alternative trees around the temporary ME tree using the topological distance (d_T) method and the bootstrap method (METREE, Rzhetsky and Nei, 1993). Using a scaling factor $f = 1$, we examined a total of 1000 bootstrap topologies. The ML phylogenies were produced using PHYLIP (Version 3.5c; Felsenstein, 1993); DNAML and DNAMLK algorithms were employed using default parameters (including a 2:1 TS/TV ratio). A statistical test of the molecular clock hypothesis for the 16S rRNA sequence was made by comparing the likelihood estimates of the DNAML and the DNAMLK trees using the

likelihood ratio test described in PHYLIP. Heterogeneity in evolutionary rates among taxa was also examined using Tajima's (1993) χ^2 -based method.

Analysis of Nuclear and Mitochondrial Distance Measures

The relationship between allozyme divergence and mtDNA 16S rRNA sequence divergence was graphically analyzed by plotting Nei's D for allozymes against 16S rRNA percentage sequence divergence computed using the gamma-corrected Kimura's two-parameter model. For comparison, values of Nei's D versus Kimura's corrected percentage sequence divergence for mtDNA cytochrome oxidase I (COI) for seven transisthmian pairs of the snapping shrimp genus *Alpheus* (Knowlton *et al.*, 1993) were superimposed. Nei's unbiased distances were also separately plotted against percentage sequence divergences of 16S rRNA TSs and TVs, respectively.

RESULTS

Phylogenetic Relationships Based on Allozymes

Allele frequencies for all polymorphic loci are available from the first author. Of the 27 putative gene loci examined (Table 2), only *LDH-1* and *MDH* were monomorphic for all species of *Centropomus* and *Lates*. Within the genus *Centropomus*, 21 loci were polymorphic within or between species. All morphological species except *C. mexicanus* were distinguished by diagnostic allozyme loci. A sample of *C. mexicanus* (9 individuals) differed from samples of its sibling species *C. parallelus* (46 individuals) in allele frequencies at two allozyme loci; however, we could not distinguish individual *C. mexicanus* from *C. parallelus* using only allozyme data.

The MP analysis yielded 20 equally most parsimonious trees. The majority-rule consensus tree is shown in Fig. 2A. Overall, only a few bootstrap values were high. Four distinct clades occurred in 66 to 93% of the trees generated in the bootstrap analysis. These clades, specified in the form (*C. undecimalis*, *C. viridis*, *C. poeyi*, *C. nigrescens*), (*C. parallelus*, *C. mexicanus*), (*C. pectinatus*, *C. medius*), and (*C. ensiferus*, *C. robalito*, *C. armatus*, *C. unionensis*), are similar to those in the morphological hypotheses proposed by Fraser (1968) and Rivas (1986) (Fig. 1); however, no support could be found for the inclusion of the species pair *C. parallelus*–*C. mexicanus* within the *C. undecimalis* clade. Two of the three geminate species proposed by Rivas (1986) (Fig. 1C) were recovered in this analysis; monophyly for the putative *C. ensiferus*–*C. robalito* pair was not supported.

The matrix of observed Nei's unbiased genetic distances between pairwise comparisons of taxa is depicted in Table 3. The lowest distance values occurred between the species pairs *C. ensiferus*–*C. robalito* and

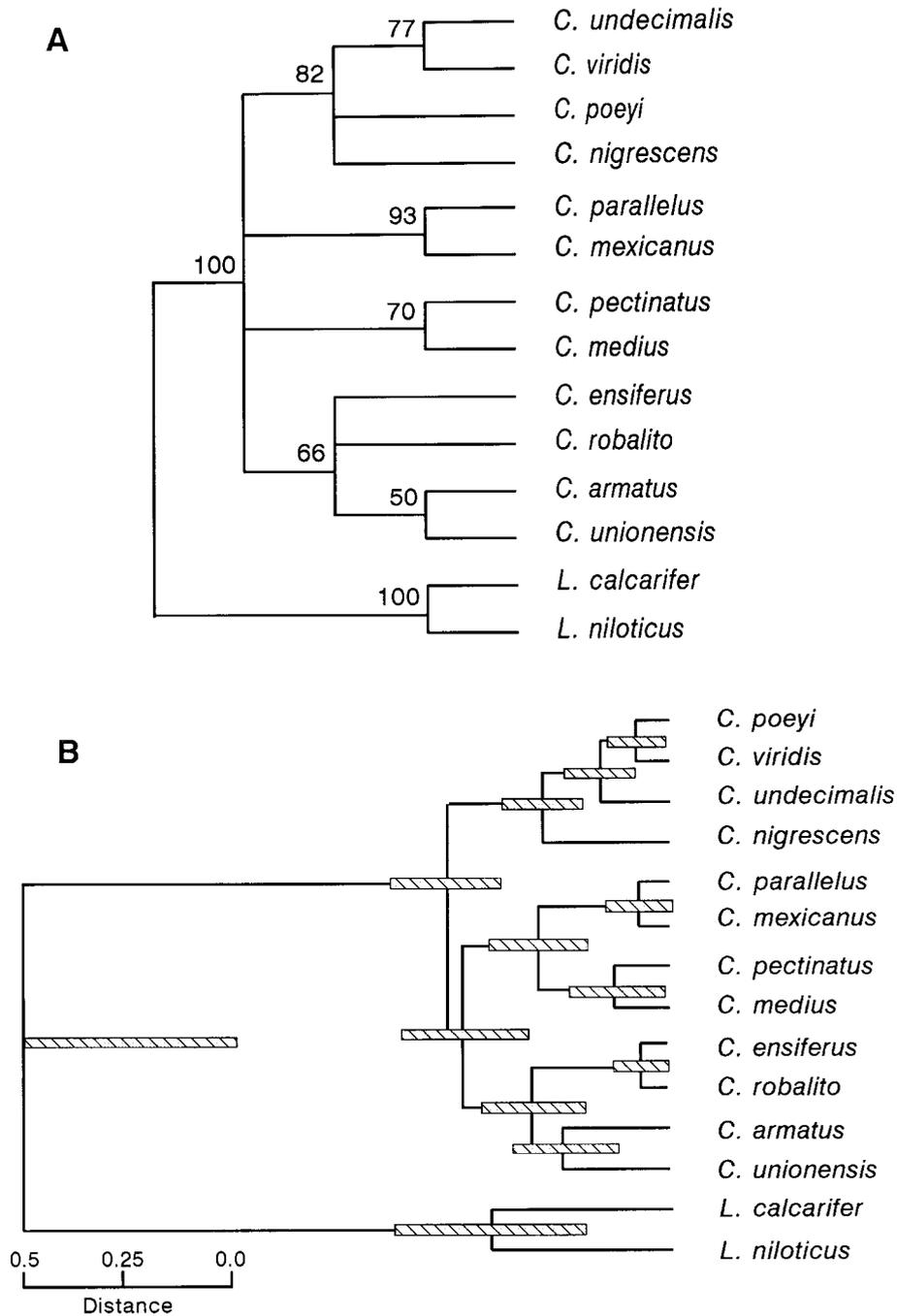


FIG. 2. Phylogenetic hypotheses for Centropominae suggested by allozyme electrophoresis. (A) 50% majority-rule consensus maximum parsimony tree (TL = 66; CI = 0.837; rescaled CI = 0.678) derived from the 20 most parsimonious trees found by PAUP 3.1.1, with bootstrap values (above the branches) based on 500 replications. (B) UPGMA phenogram based on Nei's (1978) unbiased genetic distances, with standard errors of branch lengths (hatched bars).

C. parallelus–*C. mexicanus*. The UPGMA (Fig. 2B) and NJ (not shown) analyses of these distances produced identical trees, and these were in general agreement with the majority-rule MP tree. However, standard errors at branching points on the UPGMA tree were high and the overall tree was not statistically well supported; statistical support was observed only for

generic-level subdivision and for the monophyly of the abovementioned species pairs.

Phylogenetic Relationships Based on 16S rRNA

All morphological species of *Centropomus* were unambiguously distinguished using 16S rDNA sequencing. The complete sequences used in this study were depos-

TABLE 3

Distance Matrices for *Lates* (*L.*) and *Centropomus* (*C.*) Species Based on Allozyme Allele Frequencies (above Diagonal) and mtDNA 16S rRNA Sequences (below Diagonal)

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 <i>L. niloticus</i>	—	1.34	1.36	1.35	1.10	1.19	1.22	1.10	1.09	1.47	1.33	1.25	1.46
2 <i>C. undecimalis</i>	27.4 (8.2)	—	0.08	0.12	0.24	0.45	0.54	0.49	0.40	0.65	0.32	0.52	0.57
3 <i>C. viridis</i>	25.6 (8.4)	7.9 (1.4)	—	0.05	0.14	0.34	0.43	0.50	0.40	0.59	0.32	0.53	0.51
4 <i>C. poeyi</i>	24.2 (8.0)	6.7 (1.0)	6.9 (1.6)	—	0.20	0.37	0.45	0.51	0.41	0.53	0.31	0.52	0.44
5 <i>C. nigrescens</i>	30.6 (8.3)	14.6 (3.7)	12.6 (4.1)	12.3 (3.3)	—	0.40	0.48	0.45	0.41	0.57	0.38	0.54	0.57
6 <i>C. parallelus</i>	25.8 (7.7)	12.2 (2.4)	10.3 (2.6)	11.0 (2.2)	13.1 (3.6)	—	0.04	0.22	0.18	0.41	0.39	0.30	0.35
7 <i>C. mexicanus</i>	24.6 (7.7)	11.7 (2.1)	9.1 (2.4)	11.0 (2.3)	13.6 (3.8)	2.0 (0.5)	—	0.26	0.24	0.46	0.46	0.36	0.40
8 <i>C. pectinatus</i>	26.8 (7.5)	15.9 (2.8)	13.3 (3.5)	16.9 (3.1)	17.5 (5.0)	10.2 (3.3)	10.5 (3.1)	—	0.10	0.38	0.48	0.25	0.45
9 <i>C. medius</i>	27.0 (7.2)	14.3 (2.8)	11.7 (3.0)	14.8 (2.6)	16.0 (4.3)	9.5 (2.9)	9.8 (2.8)	2.5 (0.5)	—	0.50	0.45	0.36	0.45
10 <i>C. ensiferus</i>	24.6 (7.3)	16.2 (4.5)	15.7 (4.7)	16.0 (4.7)	16.8 (6.0)	14.2 (5.2)	13.1 (5.0)	14.1 (4.5)	13.0 (4.1)	—	0.29	0.23	0.04
11 <i>C. unionensis</i>	25.8 (6.6)	16.5 (4.4)	14.1 (4.7)	16.0 (4.4)	19.9 (5.7)	16.4 (5.1)	15.4 (5.1)	15.9 (4.6)	13.8 (4.2)	8.1 (1.2)	—	0.22	0.23
12 <i>C. armatus</i>	24.4 (7.0)	15.6 (4.0)	15.0 (4.5)	16.6 (4.2)	16.3 (4.2)	13.4 (4.7)	13.1 (4.7)	11.6 (4.0)	11.1 (3.7)	4.7 (1.4)	6.1 (0.9)	—	0.28
13 <i>C. robalito</i>	26.6 (7.0)	16.4 (4.5)	17.2 (5.0)	17.7 (4.5)	17.4 (6.0)	14.2 (5.3)	13.1 (5.2)	15.2 (4.8)	14.4 (4.5)	3.6 (0.6)	9.2 (0.8)	5.8 (1.0)	—

Note. Allozyme-based distances are Nei's (1978) unbiased genetic distances. The 16S rRNA distances are gamma-corrected percentage sequence differences (Kimura's two-parameter model); values in parentheses represent uncorrected sequence divergences considering transversions only.

ited in GenBank (IntelliGenetics, Inc.) under the Accession nos. U85007–U85019. DNA samples from two to three individuals from each taxon, except for *L. calcarifer*, were sequenced (Table 1). Samples of *L. calcarifer* repeatedly failed to amplify in PCRs; therefore, that taxon was excluded from the 16S rRNA sequence analyses. Approximately 10–20% of the sequence analyzed was corroborated by reading overlapping light- and heavy-strand sequence. No base substitutions were observed within species. For the 618 sites included in this analysis, 201 (32%) were variable between taxa and 113 (18%) were informative under the conditions of parsimony.

The average frequencies of nucleotides for all taxa were as follows: A, 29%; C, 25%; G, 21%; and T, 25%. The average frequencies deviated from a hypothesis of equality (1:1:1:1; goodness-of-fit G test [Sokal and Rohlf, 1995], $G = 15.6$, $P < 0.005$, $df = 3$); however, base composition did not differ significantly among species ($R \times C$ G -test [Sokal and Rohlf, 1995]). The matrix of sequence divergences estimated using the gamma-corrected ($\alpha = 0.56$) Kimura two-parameter model is shown in Table 3. Considering both transitions and transversions, the corrected sequence divergence ranged from approximately 2 to 20% for within-*Centropomus* (ingroup) comparisons and from 24 to 27% for comparisons between *Centropomus* and *Lates* (ingroup–outgroup). Sequence divergences were lowest between the species pairs *C. parallelus*–*C. mexicanus* and *C. pectinatus*–*C. medius*. When only transversions were considered, uncorrected sequence divergence ranged from 0.5 to 6% and from 7 to 8% for ingroup and ingroup–outgroup comparisons, respectively (Table 3). In the mtDNA rRNA genes of other vertebrate species that have recently diverged (Meyer, 1993; Alves-Gomez et al., 1995), TS/TV ratios are generally high but fluctuate greatly. This trend was also observed between

pairs of closely related *Centropomus* taxa (those having sequence divergences lower than 8%); the average TS/TV ratio was 4.0 ± 1.6 . In comparisons of *Centropomus* pairs having sequence divergences greater than 8%, the average TS/TV ratio decreased ($\bar{x} = 1.8 \pm 0.6$). In all comparisons between *Centropomus* species and the outgroup, the average TS/TV ratio was 1.3 ± 0.2 . Overall, transitions generally appeared to be unsaturated in comparisons of closely related species of *Centropomus*, approached saturation in comparisons of distantly related species of *Centropomus*, and were nearly completely saturated in comparisons of *Centropomus* versus the outgroup taxon.

Weighted and unweighted MP analyses of the 16S rRNA sequences (including indels) each produced a single, identical most parsimonious tree (Fig. 3). The exclusion of indels did not alter tree topology but did slightly reduce statistical support for some nodes; bootstrap values ranged from 100 to 57 in the weighted parsimony tree not containing indels compared to a range of 100 to 62 in the weighted parsimony tree containing indels. The analyses of g_1 statistics indicated that the 16S rRNA data contain phylogenetic information, including that for the relationships at the basal portion of the tree. When we considered indels as a fifth character state, the g_1 statistics for 10,000 randomly distributed trees were -0.797 for the weighted data and -0.789 for the unweighted data. When we omitted indels, the g_1 statistics were slightly higher: -0.802 for the weighted data and -0.808 for the unweighted data. When we constrained the well-defined monophyletic taxa, as specified in the form of (*L. niloticus*, *C. undecimalis*, *C. poeyi*, *C. viridis*, *C. nigrescens*), (*C. parallelus*, *C. mexicanus*), (*C. pectinatus*, *C. medius*), (*C. ensiferus*, *C. robalito*, *C. armatus*, *C. unionensis*), the g_1 statistics for the data were -0.771 (weighted, with indels), -0.782 (unweighted, with in-

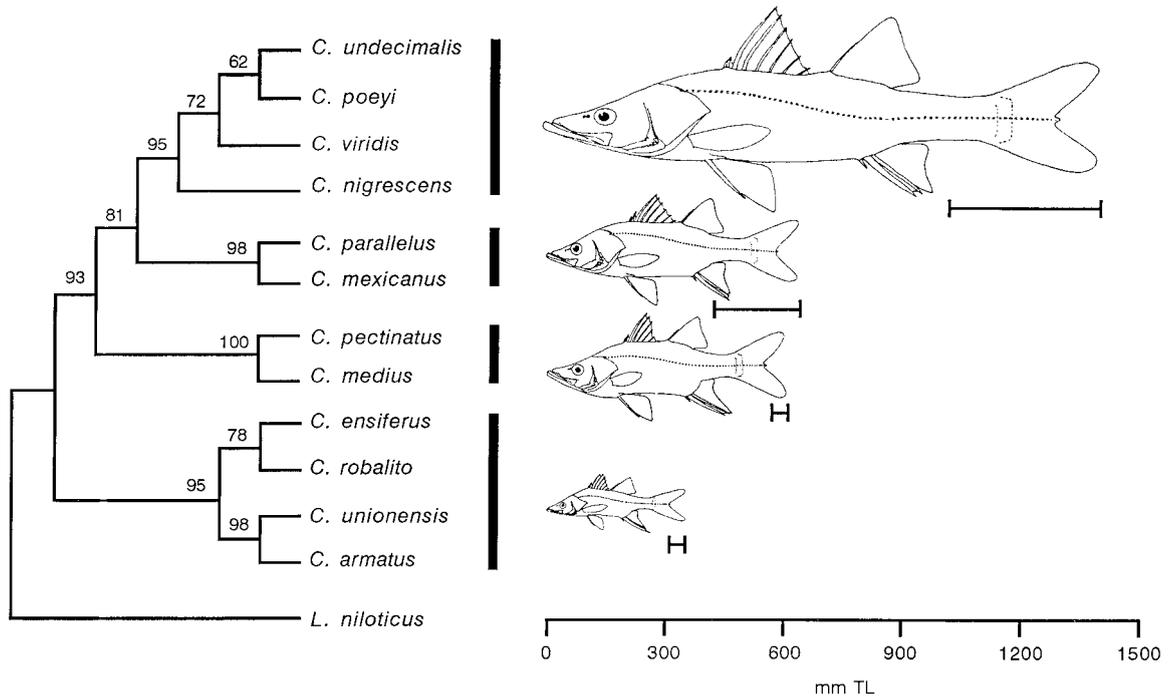


FIG. 3. Single, minimum-length, maximum-parsimony tree for Centropominae based on mtDNA 16S rRNA sequence. A single MP tree (TL = 512; CI = 0.898; rescaled CI = 0.774) was found in a branch-and-bound analysis of 618 bp (PAUP 3.1.1). Numbers on the branches indicate the percentage of trees in the bootstrap analysis (500 replications) that shared the depicted topology. Vertical bars delineate the four major clades that were supported in all analyses. Outlines of fish (redrawn from Rivas, 1986) depict the representative species of each clade (scaled to maximum reported body sizes [Table 1]); these are, from top to bottom, *C. undecimalis*, *C. parallelus*, *C. pectinatus*, and *C. ensiferus*. The horizontal bar below each drawing represents the range of maximum body sizes in mm of total length (mm TL) for all species occurring in each clade.

dels), -0.799 (weighted, without indels), and -0.804 (unweighted, without indels). All g_1 values were significant at the $P < 0.01$ level (Hillis and Huelsenbeck, 1992).

The ME analysis of the 16S rRNA data yielded a well-resolved phenogram (Fig. 4). For the gamma-

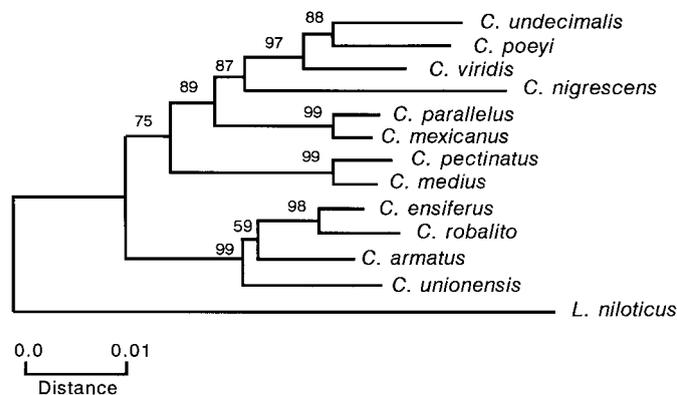


FIG. 4. Minimum-evolution tree ($S = 0.6322$) for Centropominae based on 16S rRNA sequence as reconstructed in METREE. Numbers above the branches correspond to bootstrap values (1000 replications) employing the gamma-corrected Kimura's two-parameter model. The tree topology was identical to that of the neighbor-joining tree.

corrected Kimura's two-parameter model, the NJ tree had a sum of branch lengths (S) of 0.632. The topology of the ME tree and sum of branch lengths were identical to those of the NJ tree. The ML analyses yielded tree topologies identical to those produced by the MP and ME procedures. Using the DNAML algorithm for all taxa, we obtained a log-likelihood value of -2693.5 ; all branch lengths were significantly positive at the $P < 0.01$ level. Using the DNAMLK algorithm (which assumes a molecular clock) for all taxa, we obtained a log-likelihood value of -2711.8 . The log-likelihood estimate for the tree constrained by the assumption of a molecular clock was significantly lower than was the estimate for the unconstrained tree ($\chi^2 = 36.7$, $df = 11$, $P < 0.001$). Therefore, the hypothesis of overall rate constancy among taxa was rejected for *Centropomus*.

Tajima's (1993) χ^2 -based procedure allowed us to identify the taxa responsible for the observed rate heterogeneity. This method is typically used to test the hypothesis of rate constancy between pairs of taxa within a lineage against a member of another lineage (an outgroup). Well-resolved portions of the MP and ME trees (clades with nodes having bootstrap values >95) served as the taxonomic framework for this analysis. In all three-way comparisons between two

members of the *C. undecimalis* group and an outgroup, the hypothesis of rate homogeneity could not be rejected. Similarly, in all three-way comparisons involving species other than members of the *C. undecimalis* group, substitution rates were homogeneous. However, in three-way comparisons involving one member of the *C. undecimalis* group, one member of its sister group (*C. parallelus*), and an outgroup to those taxa, the hypothesis of rate homogeneity could be rejected in all comparisons. Moreover, the hypothesis of rate homogeneity could be rejected in 20 of the 48 three-way comparisons involving one member of the *C. undecimalis* group, one member of the *C. pectinatus* group, and an outgroup to those taxa. Significant rate heterogeneity was detected in only 1 of 16 comparisons involving one member of the *C. undecimalis* group, one member of the basal *C. ensiferus* group, and the outgroup *L. niloticus*. In each of the significant comparisons, the rate of nucleotide substitution was higher than expected for the member of the *C. undecimalis* species group. This suggests that, although rates of 16S rRNA substitution are similar among members of the *C. undecimalis* group, the substitution rates of its members have been somewhat elevated relative to their congeners. Tajima's test loses statistical power in computer simulations when the outgroup for the three-way comparison is too distantly related to the ingroup taxa being compared (Tajima, 1993). This likely explains the trend toward a reduction of significant test results in comparisons between members of the *C. undecimalis* group and other species as the genetic distance between the ingroup and outgroup taxa increased.

Based on these results, we hypothesized that the substitution rates would be homogeneous among members outside of the *C. undecimalis* group. We repeated Felsenstein's likelihood ratio test after removing members of this group; the resulting log-likelihood estimate of the tree constrained by the molecular clock assumption was not significantly lower than that of the unconstrained tree. Thus, for the 16S rRNA gene, members of the *C. undecimalis* group appear to have evolved at a significantly different (faster) rate compared to the other species of snook but the molecular clock hypothesis could not be rejected for the other species.

Considering all phylogenetic reconstruction methods, a general hypothesis for Centropominae based on the 16S rRNA gene may be specified in the following form: (*L. niloticus*, (((*C. ensiferus*, *C. robalito*), (*C. armatus*, *C. unionensis*))), ((*C. pectinatus*, *C. medius*), ((*C. parallelus*, *C. mexicanus*), (*C. nigrescens*, (*C. viridis*, (*C. undecimalis*, *C. poeyi*)))))). Overall, the 16S data are robust; neither the general methods used nor the various distance corrections and weighting schemes significantly affected tree topology. The topologies of NJ and ME phenograms (e.g., Fig. 4) were nearly identical to that of the bootstrap MP phylogram (Fig. 3). The only difference between the MP tree and the NJ and ME

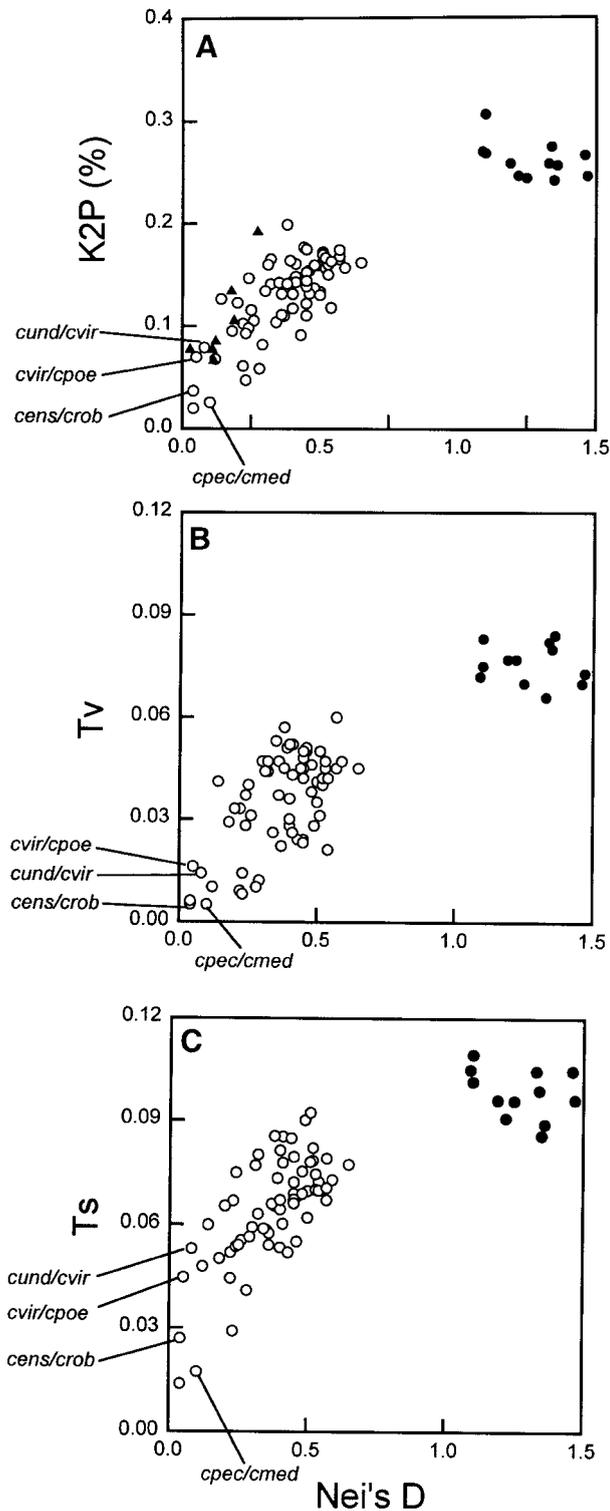
trees was that *C. unionensis* and *C. armatus* formed a clade in the parsimony analysis that was not recovered in the phenetic analyses. However, this clade was also recovered in the ML analysis, and the branch length separating it from the others was significantly positive (likelihood ratio test; $P < 0.01$).

Comparison of Allozyme and mtDNA 16S rRNA Phylogenies and Distances Measures

The hypotheses based on both the allozyme data and the mtDNA 16S rRNA sequence data indicate that the genus *Centropomus* is subdivided into four major species groups. Bootstrap values for the four clades were generally high in the 16S rRNA analyses compared to those for the allozyme data, perhaps because of the larger number of characters. In parsimony analyses, consistency indices for both data types were high (CI > 0.83). Unlike the allozyme data, the 16S rRNA data contained sufficient phylogenetic information to resolve the deeper branches of the phylogeny, thereby elucidating sister group relationships between the four major clades. These data indicate that the *C. undecimalis* group forms the sister taxon to a group containing *C. parallelus* and *C. mexicanus*. These taxa collectively form the sister group to the *C. pectinatus* group. The *C. ensiferus* group forms the basal sister taxon to the other members of *Centropomus*. There was a single incongruency between the allozyme majority-rule consensus MP tree and the 16S rRNA phylogenies—the ordering of the species triplet (*C. undecimalis*, *C. poeyi*, *C. viridis*). However, the relationships produced by the parsimony and phenetic analyses of the allozyme data were incongruent and poorly supported statistically, whereas the relationship favored by the 16S rRNA analyses (*C. viridis* ((*C. undecimalis*, *C. poeyi*))) was also recovered with good statistical support in an analysis of mtDNA cytochrome *b* gene sequence (S. Seyoum, T. Bert, and M. Tringali, unpublished data). We observed three sets of transisthmian geminate taxa in *Centropomus*. Two of these sets (*C. ensiferus*–*C. robalito* and *C. pectinatus*–*C. medius*) are species pairs and one set (*C. viridis*–*C. undecimalis*–*C. poeyi*) appears to be a species triplet in which the ancestor of *C. undecimalis* and *C. poeyi* underwent a second division subsequent to its divergence from *C. viridis*.

Relationships between allozyme and mtDNA 16S rRNA divergences for pairwise comparisons of taxa are depicted in Fig. 5. Compared to data for *Alpheus*, the larger sizes and increased number of comparisons may provide a better reflection of the amount of variance associated with comparisons of allozyme and mtDNA divergence estimates. The high variance may also be related to the observed rate heterogeneity among 16S rRNA lineages. The relationship between allozyme distance and 16S rRNA distance was not linear over evolutionary time, ostensibly due to the effects of multiple substitutions in 16S rRNA lineages (Figs. 5B

and 5C). Whereas the measures of allozyme divergence did not differ significantly between the three sets of geminate taxa, the 16S rRNA divergence values for the geminate species in the *C. undecimalis* lineage were significantly greater than the divergence values for the other two geminate pairs.



DISCUSSION

The phylogenetic hypothesis produced for *Centropomus* from the molecular genetic data is generally congruent with the informal, partial phylogenies proposed by Fraser (1968) and Rivas (1986). However, unlike the morphological hypotheses, relationships between the major species groups within the genus were resolved. The molecular data indicate that the *C. pectinatus* group is monophyletic and quite distinct from the *C. ensiferus* group, despite Greenwood's (1976) suggestion to the contrary. A notable distinction between the molecular phylogeny and the morphological phylogenies involves the status of the *C. parallelus*-*C. mexicanus* clade. In all morphological phylogenies, recognized members of this clade have been placed within the *C. undecimalis* group. Fraser (1968) relates this taxon most closely to *C. poeyi*. However, the species forming this clade lack the majority of the derived molecular character states shared by the other members of the *C. undecimalis* group, including *C. poeyi*. Moreover, members of the *C. parallelus*-*C. mexicanus* clade are very distinct genetically. Both allozyme and sequence-based genetic distances between members of the *C. parallelus*-*C. mexicanus* clade and members of the *C. undecimalis* group are as large or larger than those between members of the *C. parallelus*-*C. mexicanus* clade and members of the *C. pectinatus* group. Thus, although it most likely represents the sister group to the *C. undecimalis* group, the *C. parallelus*-*C. mexicanus* clade (henceforth referred to as the *C. parallelus* group) should be considered as having equal evolutionary status with the other species groups.

Minor discrepancies are evident in the terminal relationships between our phylogeny and those proposed by Fraser (1968) and Rivas (1986). First, the molecular data indicate that *C. ensiferus* forms a transisthmian species pair with *C. robalito*, as proposed by Rivas, rather than with *C. armatus*, as proposed by Fraser. Second, although both Fraser and

FIG. 5. Relationship between allozyme-based Nei's *D* and mtDNA 16S rRNA sequence divergence in Centropomidae. Coordinates for transisthmian geminate taxa are labeled; species abbreviations are derived from the first letter of generic name (*G*) followed by the first three letters of the species name (*spe*) and follow the form "*Gspe*" (see Table 1). (A) Plot of Nei's *D* versus gamma-corrected ($\alpha = 0.56$) Kimura's two-parameter distance for 16S rRNA sequence for pairwise comparisons of all *Centropomus* species (open circles) and for comparisons between species of *Centropomus* and *Lates niloticus* (black circles). For comparison, plots of allozyme-based Nei's *D* versus mtDNA cytochrome oxidase I (Kimura's two-parameter distance, uncorrected) for the transisthmian species pairs of the shrimp genus *Alpheus* (Knowlton *et al.*, 1993) are also depicted (black triangles). (B) Plot of Nei's *D* versus uncorrected sequence divergence for 16S rRNA transversions for pairwise comparisons of the same species pairs as depicted in (A). (C) Plot of Nei's *D* versus uncorrected sequence divergence for 16S rRNA transitions for pairwise comparisons of the same species pairs as depicted in (A).

Rivas placed *C. poeyi* outside of a *C. undecimalis*–*C. viridis* terminal pair (*C. viridis* was synonymized with *C. nigrescens* in Fraser's analysis), *C. viridis* most likely represents the sister species to the sympatric *C. undecimalis*–*C. poeyi* species pair.

Biological and Ecological Diversification within Centropominae

The mapping of biological and ecological characters onto independently derived phylogenies may be a valuable technique in the assessment of evolutionary timing and direction (Joseph and Moritz, 1993; Westneat, 1995). The robust centropomine molecular phylogeny enabled us to analyze the historical sequences of morphological and ecological changes within the genus. Perhaps the most striking morphological diversification in *Centropomus* is the wide range of maximum body sizes attained (Fig. 4). Members of the *C. ensiferus* group—the most basal lineage—are small-bodied species. They have a maximum reported size of 370 mm total length (TL) and weigh no more than 0.5 kg. Maximum sizes increase somewhat in the *C. parallelus* and *C. pectinatus* groups (630 mm TL, ~4 kg; and 608 mm TL, ~1.5 kg, respectively). However, the most significant gains in length and weight have been made by members of the *C. undecimalis* group (>1000 mm TL, 15–35 kg). Members of this group are the most nested within the rooted phylogeny. Thus, the principal pattern of evolutionary change in the genus appears to be that of an increase in overall body size.

Snook also show important ecological divergences in the salinity regimes preferred as adults. As a group, members of the genus *Centropomus* have been described as euryhaline, semicathadromous, and/or estuarine dependent (Chávez, 1963; Rivas, 1986). Fresh or extremely low-salinity or oligohaline waters reportedly constitute a habitat requirement for early stage juveniles of all snook species (Gilmore *et al.*, 1983; Peters, 1993; Muhlia-Melo *et al.*, 1995). *C. ensiferus* prefers fresh or very low-salinity water during all life stages (Muhlia-Melo *et al.*, 1995). The remaining members of the *C. ensiferus* group and the members of the *C. pectinatus* and *C. parallelus* groups are restricted solely to locations in close proximity to permanent freshwater outflow (rivers, estuaries, embayments [Rivas, 1986]); they are not known to venture into the nearshore marine or oceanic environment. In contrast, adult members of the *C. undecimalis* group often occur in locations having little or no freshwater habitat. For example, large congregations of adult *C. undecimalis* occur in the Florida Keys (Marshall, 1958), the Bahamas (Böhlke and Chaplin, 1968), and the Cayman Islands (Rivas, 1986); they also occur on coral reefs (T. Bert, pers. obsn.) and on rock outcrops in the open ocean (R. Taylor, pers. comm.). In the eastern Pacific, fishermen target coastal oceanic environments for adult *C. viridis* and *C. nigrescens*. In addition, a population of

C. viridis occurs in the Galapagos Islands (Rivas, 1986), which has never been in contact with a continental land mass. In the Gulf of Mexico, adult *C. poeyi* reportedly reside on reefs located 120 km from land (Muhlia-Melo *et al.*, 1995). In general, there appears to be an evolutionary trend in the preference of adult *Centropomus* for saltwater habitat grading from low in basal species to high in the most nested species.

All centropomines (and most latines) are capable of becoming isotonic at full seawater salinities (Dunstan, 1959; Chung, 1981), allowing them to withstand the sharp and recurrent shifts in estuarine salinities (Odum, 1953). However, only members of the *C. undecimalis* group routinely traverse into and inhabit the open ocean and coastal marine waters. Larger body sizes (or faster rates of growth) and gains in swimming speed due to increased size (Webb, 1975) may afford members of the *C. undecimalis* group both a wider range of prey size and a greater ability to avoid predators (Holbrook and Schmitt, 1979); factors important to niche expansion (Holbrook and Schmitt, 1979; Werner, 1979). In comparison to those of estuarine and marine systems, the faunal composition of neotropical rivers throughout most of the centropomine distribution contains a narrower size range of potential prey items. Adult members of the small-bodied snooks generally prey upon smaller fishes such as engraulids and gobiids as a supplement to a diet that is principally based on crustaceans. In contrast, prey items for adult members of the *C. undecimalis* group include mugilids, leiognathids, sciaenids, and sparids (Chávez, 1963), i.e., larger estuarine and coastal marine fishes. Thus, snook species are partitioned along both prey and distribution gradients that appear to be unidirectionally reinforcing.

Molecular Clocks and Rates of 16S rRNA Divergence in Centropominae

Because the emergence of the Panamanian isthmus has been implicated in the speciation of three of the terminal groups of centropomine taxa (Rivas, 1986), the potential exists to explore evolutionary rates in the genus. Having a robust molecular phylogenetic hypothesis and knowledge of the disparate life histories within the genus facilitates such an investigation. Additional confidence in this exercise may be derived from the association between 16S rRNA and allozyme divergences over time, which suggests that snook generally evolved in a "clocklike" manner. However, because measures of allozyme and 16S rRNA divergence for the three sets of putative geminate taxa were discordant, the estimation of 16S rRNA divergence rates for *Centropomus* is complicated (Bermingham and Lessios, 1993). In particular, 16S rRNA divergence values among the three sets of geminate species differed significantly. As indicated by the relative rate test, the differing amounts of 16S rRNA divergence could be the result of an

accelerated substitution rate in the *C. undecimalis* group.

Substitutions in the 16S rRNA gene were gained or became fixed at a faster relative rate in the geminate taxa in the *C. undecimalis* group compared to geminate species of other groups. The hypotheses suggesting that long-lived, large-bodied species or species with long generation intervals should have slower rates of mutation compared to small-bodied, shorter-lived species or species with short generation intervals (Britten, 1986; Li and Tanimura, 1987) do not seem to apply to snook. Members of the *C. undecimalis* group grow much larger and are up to 70 times heavier than members of the other groups. Members of the *C. undecimalis* group also commonly live 2–3 times longer than the oldest reported snook from any other group (Fuentes-Castellanos, 1973; Taylor *et al.*, 1993; J. Whittington, unpublished data). The available reproductive data for Atlantic species (reviewed by Muhlia-Melo *et al.*, 1995) indicate that females of large-bodied snook attain reproductive maturity at older ages than do females of small-bodied snook. Therefore, the direction of increase in 16S rRNA substitution rates among centropomine species was, in fact, opposite to that predicted by the “metabolic-rate” and “generation-interval” hypotheses.

In contrast to the above hypotheses, any of the hypotheses that pertain to influences on fixation rates (e.g., differences in the amount of cladogenesis [Futuyma, 1987], effective population sizes [DeSalle and Templeton, 1988], or intensities of purifying or directional selection [reviewed by Mindell and Thacker, 1996]) may apply to snook. In *Centropomus*, there is no evidence favoring one of these hypotheses over another.

Alternatively (but not mutually exclusive of rate acceleration), the speciation of the *C. undecimalis* transisthmian pair may have occurred prior to the speciation of the other transisthmian pairs. This scenario is not supported by measures of allozyme divergence. However, it is generally supported by the degree of relative morphological divergence exhibited in the geminate taxa (Rivas, 1986) and by the pattern of 16S rRNA transversional changes between geminate species. Since transversions are thought to accumulate linearly over time and more slowly than transitions (Meyer, 1993), divergence rates of transversions in mtDNA rRNA genes (typically ~0.15%/my) have been used preferentially to estimate divergence times among taxa, including divergence times among species and genera of perciform fishes (Ritchie *et al.*, 1996). Assuming that isolation between Pacific and Atlantic populations occurred approximately 3 mya, the rate of transversional divergence between *Centropomus* transisthmian geminate taxa (excepting members of the *C. undecimalis* group) is approximately 0.2%/my. If transisthmian taxa in the *C. undecimalis* group also diverged at that time, their rate of transversional change would be 0.5%/my—a rate that is unprecedented in

vertebrate evolution (Kraus and Miyamoto, 1991; Caconne *et al.*, 1994). Thus, notwithstanding 16S rates that may be somewhat elevated in the *C. undecimalis* group, it appears that the transisthmian taxa in that group may have diverged several million years before the other geminate taxa.

Because of the observed acceleration in the evolutionary rate of 16S rRNA and possible early divergence, geminate species of the *C. undecimalis* group were excluded from further analysis of 16S divergence rates. Instead, we used the two sets of transisthmian pairs that had similar evolutionary ages and that exhibited rate constancy with the majority of the snook species (*C. pectinatus*–*C. medius* and *C. ensiferus*–*C. robalito*) to establish a general rate of 16S divergence for the genus. Assuming that the final closure of the Panama Seaway isolated Atlantic- and Pacific-inhabiting ancestral populations of these two species pairs approximately 3 mya (Coates *et al.*, 1992), we estimate divergence rates between the 16S rRNA genes of the geminate species for the two species pairs to be approximately 0.8%/my (range: 0.6–1.1%/my) and 1.2%/my (range: 0.9–1.5%/my), respectively.

Divergence rates for *Centropomus* are within the reported range of 16S ribosomal genes of endothermic vertebrates (reviewed in DeSalle *et al.*, 1987) but are faster than those reported for other ectothermic vertebrates (Martin *et al.*, 1992). Although estimates of 16S rRNA divergence rates in other teleost fishes are not available for comparison to snook, rate estimates for mtDNA protein-coding genes are. For example, Birmingham *et al.* (1997) reported average divergence rates of 1.2%/my for mtDNA COI and 1.3%/my for NADH dehydrogenase subunit 2 and ATPase6 genes between other pairs of transisthmian marine teleosts. In general, mitochondrial ribosomal genes show less sequence divergence than do mitochondrial protein-coding genes (Meyer, 1993). Therefore, evolutionary rates estimated for snook 16S ribosomal genes appear to be reasonable when compared to rate estimates derived for mtDNA protein-coding genes of teleost fishes. Recognizing the high variances surrounding the rate estimates, an overall 16S rRNA divergence rate of approximately 1%/my and a 16S rRNA transversion divergence rate of 0.2%/my could be used to infer the general timing of divergence events for other centropomine taxa. Because of apparent saturation effects on divergence estimates between distant taxa, use of the transversion divergence rate may result in more accurate estimates of divergence times at deeper branches; but even this rate should not be extended to phylogenetic events beyond the generic level.

The empirically derived rate estimates may be used in conjunction with proposed land bridge models to examine the temporal biogeographic framework of the recent diversification of *Centropomus*. Applying the 16S transversion divergence rate, estimated diver-

gence times suggest that the four major divisions within the genus and the associated ecological differences were in place by the mid- to late Miocene (~10 mya). Divergence times may also indicate that the transisthmian pairs in *Centropomus* did not speciate by way of a single vicariant mechanism. The period preceding the final Pliocene emergence of the land bridge was marked by a series of fluctuations in the geography and physical characteristics of the associated coastal and estuarine environments (Coates *et al.*, 1992). Based on divergence times of freshwater fishes that colonized the Atlantic drainages of western Panama and Costa Rica, Bermingham and Martin (1998) postulated that reduced sea levels and the coincident uplift of the Chorotega block at the close of the Miocene 5–7 mya may have connected the continental land masses and provided sufficient emergent landscape for a pre-Pliocene invasion of South American primary freshwater fishes into North America. The subsequent inundation of lower Central America at the start of the Pliocene presumably caused drastic changes in faunal assemblages and the extirpation of many of the pre-Pliocene colonizing freshwater species, especially those unable to exist in the shoal environments characteristic of the region at that time. Thus, it is interesting that in both snook and snapping shrimp (Knowlton *et al.*, 1993) there are some species pairs whose divergences are tightly associated with the final closure of the land bridge and some species pairs having earlier divergence times. For snook, the transisthmian species pairs whose life histories are more closely tied to freshwater habitat appear to have diverged around the time of the final Pliocene land bridge emergence, whereas the pairs having a significant marine component to their life histories appear to have diverged around the time of the late Miocene closure. Similarly, the transisthmian clades of marine species of *Alpheus* seem to have been formed prior to transisthmian clades of nearshore species. Accordingly, as postulated for *Alpheus* (Knowlton *et al.*, 1993), ecological diversification among the various ancestral snook species appears to be reflected in the timing of their evolutionary divergences.

For *Centropomus*, deeper sister group relationships not yet evident from species morphology were resolved using molecular data. The overall association between allozyme and 16S rRNA divergences supports the general clocklike nature of snook evolution, and two important evolutionary changes in *Centropomus* have resulted in considerable ecological diversification among extant snook species. As may be the general case for neotropical marine and estuarine organisms, vicariant events associated with the geological evolution of the Central American isthmus undoubtedly made a strong contribution to the species diversity present in *Centropomus*. In addition, the disparate life histories and ecologies of ancestral centropomine species seem to have played a role in the speciation process by influenc-

ing the species' response to the dramatically changing Neogene environments in the Central American tropics.

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