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Short Communication

A mitochondrial DNA based phylogeny of weakfish species of the *Cynoscion* group (Pisces: Sciaenidae)

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ABSTRACT

We infer the phylogeny of fishes in the New World *Cynoscion* group (*Cynoscion, Isopisthus, Macrodon, Atractoscion, Plagioscion*) using 1603 bp of DNA sequence data from three mitochondrial genes. With the exception of *Plagioscion*, whose position was ambiguous, the *Cynoscion* group is monophyletic. However, several genera examined are not monophyletic. Atlantic and Pacific species of *Cynoscion* are interspersed in the tree and geminate species pairs are identified. Intergeneric relationships in the group are clarified. Our analysis is the first comprehensive phylogeny for the *Cynoscion* group based on molecular data and provides a baseline for future comparative studies of this important group.

1. Introduction

Cynoscion is a large, ecologically and economically important genus of marine fishes in the family Sciaenidae found throughout tropical and subtropical coastal waters of the New World. Twenty-four species known as corvinas or weakfish are currently recognized. *Cynoscion* are important predators in coastal ecosystems and have relatively streamlined, elongate bodies, large mouths and sharp teeth, including well-developed canines. They are most common in shallow, coastal environments and estuaries, although some occur in fresh water or in deep ocean waters (e.g., Béarez, 2001). The genus is highly valued as a human food source and is actively exploited throughout its range (Chao, 1995, 2002).

Despite its ecological and economic importance, the phylogeny of the genus *Cynoscion* has not been resolved. Previous studies have investigated the phylogenetic relationships among regional assemblages (Weinstein and Yerger, 1976; Paschall, 1986; Aguirre, 2000; Vinson et al., 2004), and only one study, based on otolith morphology, has evaluated phylogenetic relationships of the entire genus (Schwarzhans, 1993). The intergeneric relationships of *Cynoscion* are also unclear. Based on morphological traits, Chao (1978) and Sasaki (1989) placed *Cynoscion Macrodon*, *Isopisthus* and tentatively the South American freshwater genus *Plagioscion* together in the *Cynoscion* group. *Atractoscion* has also been suggested to be phylogenetically close to *Cynoscion* based on morphological similarities (e.g., Trewavas, 1977; Sasaki, 1985; Schwarzhans, 1993).

In this paper, we infer a phylogenetic hypothesis for the genus *Cynoscion* and closely related genera using mitochondrial DNA sequence data.

2. Materials and methods

Sample sizes and locations are listed in Table 1 and Fig. 1. Tissue samples were frozen in liquid nitrogen or were preserved in DMSO/EDTA buffer or 95% ethanol. Taxonomic sampling of the *Cynoscion* group included 19 of the 24 *Cynoscion* species currently recognized (Eschmeyer, 1998), and species of *Isopisthus, Macrodon, Plagioscion*, and *Atractoscion*. The species of *Cynoscion* for which we could not obtain tissue samples are *C. similis, C. steindachneri, C. nannus, C. nortoni* and *C. stolzmanni*. Other sciaenids used as outgroups included species of *Bairdiella, Nebris, Seriphus*, and *Stellifer*.

DNA was extracted using standard CTAB/phenol/chloroform techniques (Sambrook et al., 1989) or QIAamp DNA purification kits (QIAGEN, Inc.). The ATP synthase 6 and 8 (ATPase 8/6) genes were PCR amplified using primers L8331 (5′-AAAGCRTYRGCCTTTT

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Table 1
Mitochondrial DNA phylogeny of *Cynoscion*. Species, sample sizes and collecting locality.

Species	Sample size	Collecting locality
Cynoscion albus (Calb)	4	Gulf of Panamá (Pacific)
Cynoscion phoxoxephalus (Cphx)	6	Gulf of Panamá (Pacific)
Cynoscion squamipinnis (Csqu)	5	Gulf of Panamá (Pacific)
Cynoscion reticulatus (Cret)	2	Gulf of Panamá (Pacific)
Cynoscion praedatorius (Cprd)	2	Gulf of Panamá (Pacific)
Cynoscion xanthulus (Cxth)	2	Gulf of California (Pacific)
Cynoscion othonopterus (Coth)	2	Gulf of California (Pacific)
Cynoscion parvipinnis (Cpar)	2	Gulf of California (Pacific)
Cynoscion analis (Cana)	2	Gulf of Guayaquil (Pacific)
Cynoscion jamaicensis (Cjam)	2	Maracaibo Lake (Atlantic)
Cynoscion leiarchus (Clei)	2	Maracaibo Lake (Atlantic)
Cynoscion microlepidotus (Cmic)	2	Maracaibo Lake (Atlantic)
Cynoscion virescens (Cvir)	2	Maracaibo Lake (Atlantic)
Cynoscion acoupa (Caco)	3	Maracaibo Lake (Atlantic)
Cynoscion regalis (Creg)	4	Chesapeake Bay, Virginia (Atlantic)
Cynoscion nebulosus (Cneb)	2	Chesapeake Bay, Virginia (Atlantic)
Cynoscion arenarius (Care)	3	Gulf of México, Texas (Atlantic)
Cynoscion nothus (Cnot)	2	Gulf of México, Texas (Atlantic)
Cynoscion guatucupa (Cgua)	3	Estuary of Río de La Plata (Atlantic)
Isopisthus remifer (Irem)	2	Gulf of Panamá (Pacific)
Isopisthus altipinnis (Ialt)	2	Maracaibo Lake (Atlantic)
Macrodon mordax (Mmor)	2	Gulf of Panamá (Pacific)
Macrodon ancylodon (Manc)	2	Maracaibo Lake (Atlantic)
Plagioscion squamosissimus (Psqu)	2	Cuyabeno River, Perú (Pacific)
Nebris occidentalis (Nocc)	2	Gulf Panamá (Pacific)
Nebris microps (Nmic)	2	Maracaibo Lake (Atlantic)
Bairdiella armata (Barm)	1	Parita Bay, Panamá (Pacific)
Bairdiella ronchus (Bron)	1	Colón, Caribbean Panamá (Atlantic)
Seriphus polithus (Spol)	2	Southern California (Pacific)
Stellifer illecebrosus (Sill)	1	Parita Bay, Panamá (Pacific)

AAGC-3′) and H9236 (5′-GTTAGTGGTCAKGGGCTTGGRTC-3′) (Perdices et al., 2002). In addition, the cytochrome *b* (cyt *b*) gene was PCR amplified with the primers GluDG.L (5′-TGACCTGAARAACCA YCGTTG-3′) (Palumbi, 1996) and H16460 (5′-CGAYCTTCGGATTACA AGACCG-3′). In both genes double-stranded DNA was PCR synthesized in 25 μl reactions (2.5 μl 10 mM Tris–HCl buffer, 2 μl 2.0 mM $MgCl_2$, 1.5 μl 10 mM of each primer, 2.5 μl dNTP 200 nM/dinucleotide, 1 μl template DNA, and 0.25 μl [1U] Amplitaq polymerase [Perkin-Elmer]). Thermocycler conditions were: preheat at 94 °C for 120 s (seconds), denaturation 94 °C for 45 s, annealing 53 °C for 45 s, extension 72 °C for 90 s, repeated for 5 cycles, followed by 29 cycles at 94 °C for 45 s, 58 °C for 45 s and 72 °C for 90 s. PCR product were sequenced using the two amplification primers and one internal primer, Cb3H (5′ GGCAAATAGGAARTATCATTC 3′; Palumbi, 1996) for the cyt *b* gene, and 8.3 (5′ TGATAKGCRTGT GCTTGGTG 3′) for the ATPase 8/6 gene. The PCR products were electrophoresed in 1.5% low melting point agarose gels using a Tris–acetate buffer (pH 7.8) containing 1 μg/ml of ethidium bromide. The single amplification product was cut from the gel and digested with 1 μl Gelase (Epicentre Technologies) at 70 °C for 120 s, followed by overnight incubation at 45 °C. Five microliters of a purified PCR product was used as template in a 10 μl cycle sequencing reaction using a dRhodamine terminator cycle sequencing kit (PE Applied Biosystems). Cycle sequencing conditions were: preheat at 96 °C for 60 s, 96 °C for 15 s, 50 °C for 1 s, and 60 °C for 4 min repeated for 25 cycles. The cycle sequencing product volume was doubled by the addition of 10 μl ddH20 and purified using Centrisep columns with 750 μl G-50 Sephadex. Samples were dried and resuspended with 5:1 blue dextran/EDTA (pH 8.0) and formamide.

We used the computer program Sequencher 4.1 (GeneCodes) to align the DNA sequences. Congruence among tree topologies of the two mitochondrial genes was tested using a Shimodaira and Hasegawa test implemented in PAUP (100 replicates). The aligned data were analyzed using maximum parsimony, maximum likelihood, and Bayesian methods. The maximum parsimony and maximum likelihood analyses resulted in topologies that were statistically equivalent (S–H test, $p = 0.5$) and similar to the Bayesian consensus tree, so we only present the results of the Bayesian analysis.

Bayesian phylogenetic analyses were performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) through the Computational Biology Service Unit at Cornell University. Preliminary runs were performed to monitor the fluctuating value of the likelihoods of the Bayesian trees, and all parameters appeared to reach stationarity before 250,000 generations (stationarity was achieved when the log-likelihood values of the sample points reached a stable equilibrium value). The Markov chain analysis was run for 5 million generations and trees were sampled every 500 generations. The analyses were run employing six simultaneous chains starting with a random tree. A burn-in period, in which the initial 2000 trees were discarded, was adopted and the remaining tree samples were used to generate a strict consensus tree. The posterior probability of each clade is provided by the percentage of trees identifying the clade; posterior probabilities $\geqslant 0.95$ are considered significant support.

3. Results

Complete sequences were obtained for the ATPase 8/6 genes (842-bp) and the 5′-end portion of the cytochrome *b* gene (761-bp) for 51 individuals belonging to 19 species of *Cynoscion* and from one or two individuals of 12 other sciaenid species (Fig. 2; GenBank Accession No.: ATPase 8 GQ219964-GQ219994, cyt *b* GQ219995-GQ220025, ATPase 6 GQ220026-GQ220056). None of the sequences exhibited stop codons when translated to amino acids. There was no evidence of nucleotide saturation when transitions and transversions were plotted against uncorrected genetic distances so all nucleotide positions were used. Plots of the complete sequence for each gene or by codon position were linear (data not shown). Third position transitions accumulated more quickly and the cyt *b* gene exhibited somewhat higher substitution rates than the ATPase 8/6 genes. The Kimura 2-parameter genetic distances ranged from 0.3% (*C. albus* and *C. acoupa*) to 14.3% (between *C. leiarchus* and *C. virescens*) to 24.5% (*Macrodon mordax* and *B. ronchus*) (Table 2). Overall phylogenetic relationships using ATPase 6/8 and cyt *b* markers did not differ significantly (Shimodaira–Hasegawa test, $p = 0.25$), thus the two datasets were combined and analyzed together.

The Bayesian inference analysis of combined data resulted in a 95% credible set of 2827 trees and the consensus tree is presented in Fig. 2. Resolution at the tips was good while resolution deep in the tree was more problematic. Pacific and Atlantic species of *Cynoscion* are interspersed in the tree with several apparent geminate species pairs possibly associated with the rise of the Isthmus of Panama. Monophyly of the *Cynoscion* group including the genera *Isopisthus*, *Macrodon*, and *Atractoscion* is well supported. The relationship between *Plagioscion* and the *Cynoscion* group is ambiguous, however, because *Plagioscion* fell out between the *Cynoscion* group and the outgroup genera. Monophyly of genera as presently recognized was not supported. *Isopisthus* occurs within the genus *Cynoscion, Atractoscion nobilis* is the sister taxon to *C. guatucupa*, and *C. virescens* is the sister taxon to *Macrodon*. Many internode distances in the trees are small suggesting that divergence occurred quickly at several stages in the history of the lineage (Table 2), impeding resolution of phylogenetic relationships of all lineages.

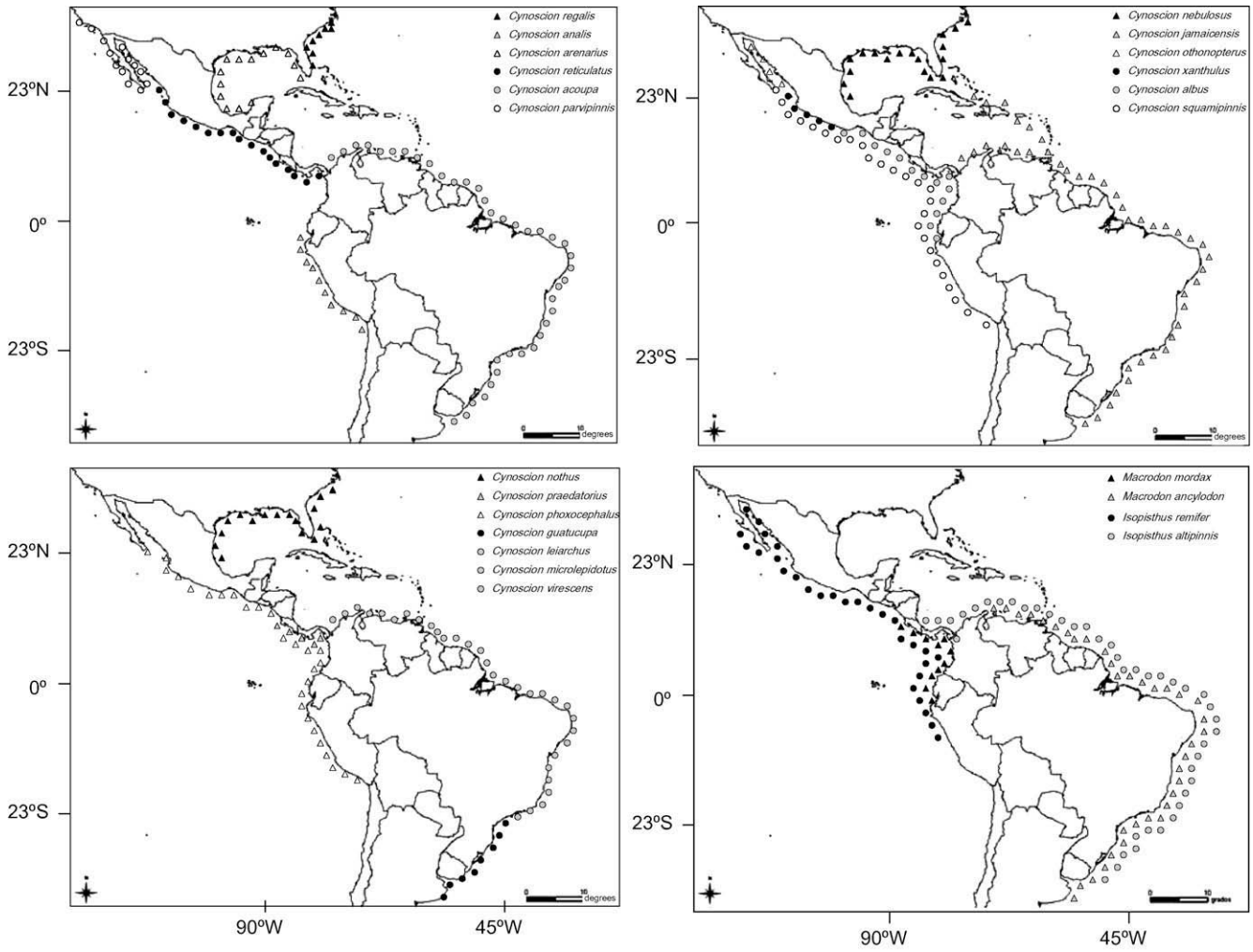

Fig. 1. Maps showing distribution of *Cynoscion* species and related genera used in this study.

4. Discussion

Cynoscion is a highly diverse genus of mostly large, coastal predatory fish, rivaled in species richness among New World sciaenids only by the genus *Stellifer*. All *Cynoscion* species are restricted to either the Atlantic or Pacific and species do not segregate into clades by ocean; Atlantic and Pacific species are interspersed in the phylogeny (Figs. 1 and 2). The latitudinal ranges of species also vary considerably with a particularly strong break between northern and southern species occurring in the Atlantic (Caribbean). This pattern suggests that speciation in *Cynoscion* has been facilitated by vicariance events between the two continents and two ocean basins, as previously suggested by Sasaki (1989). Lack of resolution among taxa at the base of the *Cynoscion* group may reflect rapid speciation events during which there was insufficient time for the accumulation of shared derived substitutions.

Our results pointed to several geminate species relationships between Atlantic and Pacific species of the *Cynoscion* group including *C. reticulatus/C. nothus, C. phoxocephalus/C. leiarchus, Macrodon mordax/M. ancylodon,* and *Isopisthus remifer/I. altpinnis.* The relationship between *I. altipinnis, I. remifer,* and *C. analis* is not well resolved, but we consider *I. altipinnis* and *I. remifer* likely sister species because of their morphological similarity. The rise of the *Isthmus* of Panama in the mid Miocene is known to have been a major source of biological diversity in the region and may have played a role in the origin of these four pairs of geminate species. We also observed three closely related pairs of sister species in the genus *Cynoscion* occurring in the same ocean, *C. regalis/C. arenarius* (Western Atlantic), *C. praedatorius/C. squamipinnis* and *C. albus/C. xanthulus* (the two latter in the Eastern Pacific). The first two sets of species are largely allopatric (Fig. 1), suggesting an allopatric origin. In the last species pair, *C. praedatorius* occupies a subportion of the range of *C.* squamipinnis (Fig. 1), suggesting that *C. praedatorius* evolved from isolated *C. squamipinnis* populations in Central America.

The relationship between the genera *Cynoscion*, *Isopisthus, Macrodon, Atractoscion,* and *Plagioscion* had been examined previously using morphological traits (Chao, 1978; Sasaki, 1989). Our molecular analysis supports some of the previous phylogenetic hypotheses but clearly refutes others. Species in the genus *Isopisthus* (*I.* remifer and *I. altipinnis)* fall solidly within the genus *Cynoscion*, as suggested previously by Aguirre (2000) based on 12S/16S rRNA mtDNA sequences. This relationship is also supported by the morphological similarity between *Isopisthus* and *C. analis. Isopisthus* is easily distinguished from typical *Cynoscion* by having a highly divergent body shape, a greatly expanded anal fin and a gap between the first and second dorsal fins (Chao, 1995; Aguirre and Shervette, 2005). However, *Cynoscion analis* is morphologically intermediate between typical *Cynoscion* and *Isopisthus* (e.g., Schwarzhans, 1993; Aguirre and Shervette, 2005), suggesting that *Isopisthus* evolved from *Cynoscion*, with *C. analis* corresponding to an intermediate form. Thus, placement of the two species of *Isopis-*

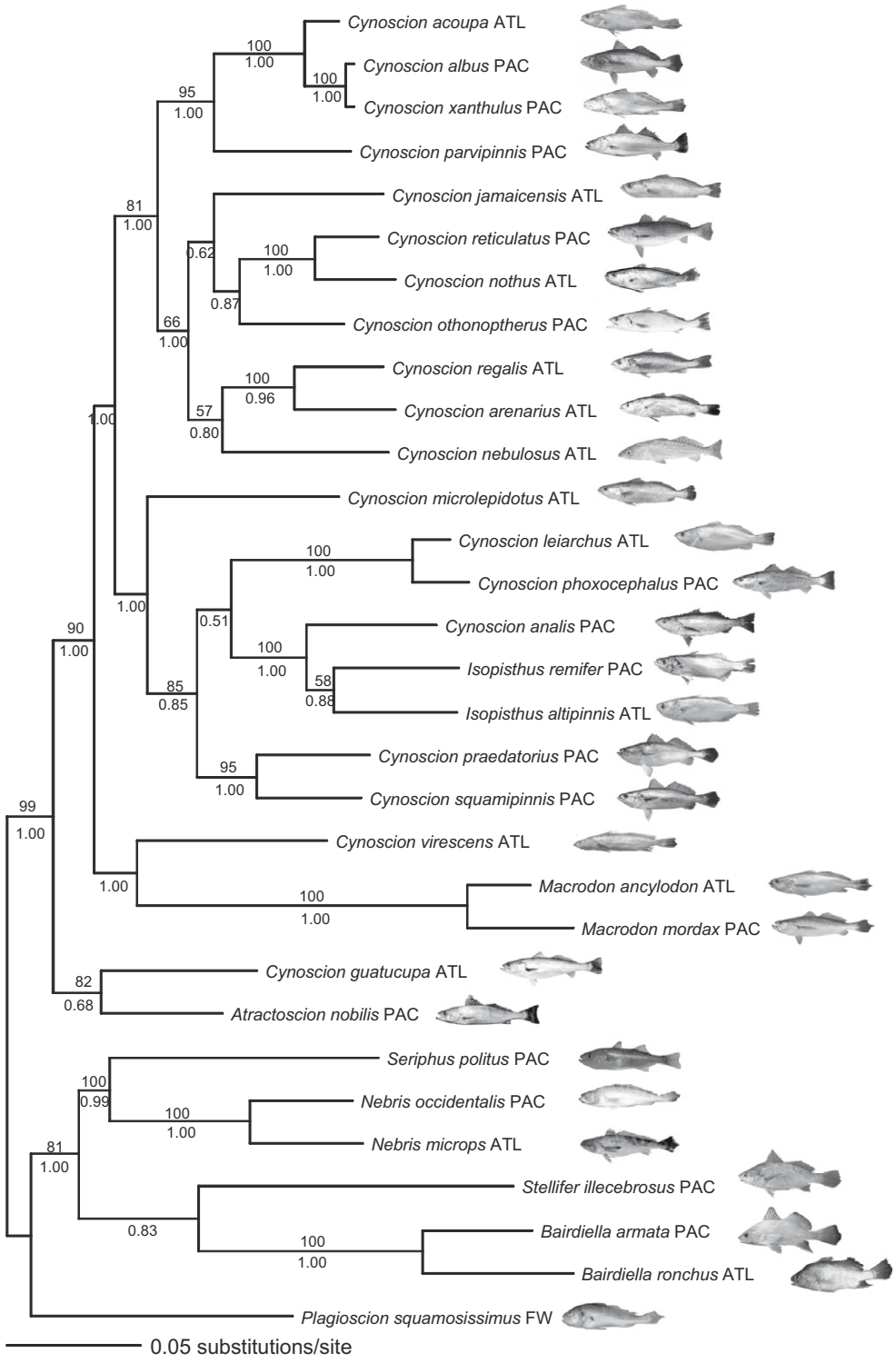

Fig. 2. Strict consensus tree from the Bayesian analysis of combined ATPase 8/6 and cyt *b* mitochondrial gene sequences. Numbers below branches are Bayesian posterior probabilities and numbers above branches are bootstrap values calculated from the maximum likelihood analysis of 1000 sequence replicates assuming model parameters values estimated from Modeltest (GTR + I + G model).

thus in a separate genus is not warranted and we suggest that they be included in the genus *Cynoscion*. *Macrodon* is likely the sister genus to *Cynoscion* (including *Isopisthus* and excluding *C. guatucupa,* see below). Although, *Macrodon* falls out with *C. virescens,* bootstrap support is not strong (<50). Given its morphological distinctiveness (*Macrodon* has conspicuous arrow-shaped teeth) and basal position in the tree, we propose that *Macrodon* be retained as a distinct genus that is the sister to *Cynoscion*, and that *C. virescens* be recognized as a primitively derived species of *Cynoscion*, pending further analysis. *Atractoscion* is the likely sister taxon to the clade consisting of the genera *Macrodon* and *Cynoscion* (including *Isopisthus*) as suggested by previous morphological analyses (Trewavas 1977; Sasaki, 1985, 1989). The genus *Atractoscion* consists of two species that occur in temperate waters of the

Table 2
Genetic divergences between 18 species of *Cynoscion*, two species of *Macrodon*, two species of *Isopisthus*, plus outgroup species based on combined dataset of mitochondrial genes: ATPase 8/6 and cytochrome *b*. The values are sequence divergence percentage between species pairs using Kimura's 2-parameters distances.

	Caco	Cjam	Cmic	Cvir	Clei	Cpar	Calb	Cxth	Creg	Care	Cret	Cnot	Coth	Cprd	Csqu	Cphx	Cgua	Cana	Cneb	Manc	Mmor	Irem	Ialt	Anob	Spol	Psqu	Nocc	Nmic	Sill	Barm
Cjam	0.115																													
Cmic	0.124	0.130																												
Cvir	0.114	0.128	0.126																											
Clei	0.135	0.139	0.127	0.143																										
Cpar	0.088	0.121	0.128	0.132	0.141																									
Calb	0.031	0.123	0.120	0.120	0.133	0.091																								
Cxth	0.031	0.124	0.121	0.119	0.133	0.091	0.006																							
Creg	0.098	0.115	0.130	0.133	0.133	0.108	0.110	0.109																						
Care	0.109	0.118	0.124	0.135	0.134	0.113	0.113	0.114	0.068																					
Cret	0.110	0.102	0.119	0.125	0.135	0.108	0.114	0.116	0.105	0.111																				
Cnot	0.111	0.108	0.117	0.125	0.137	0.115	0.116	0.114	0.109	0.114	0.052																			
Coth	0.100	0.090	0.124	0.124	0.131	0.106	0.101	0.100	0.096	0.100	0.082	0.085																		
Cprd	0.117	0.124	0.121	0.125	0.125	0.125	0.119	0.117	0.122	0.125	0.125	0.125	0.110																	
Csqu	0.121	0.127	0.120	0.117	0.114	0.122	0.118	0.114	0.127	0.126	0.122	0.119	0.113	0.077																
Cphx	0.142	0.145	0.130	0.145	0.034	0.144	0.138	0.136	0.139	0.143	0.137	0.135	0.133	0.127	0.117															
Cgua	0.135	0.134	0.122	0.130	0.129	0.131	0.137	0.138	0.127	0.115	0.123	0.126	0.119	0.130	0.125	0.139														
Cana	0.129	0.124	0.132	0.130	0.128	0.138	0.134	0.134	0.123	0.135	0.131	0.130	0.124	0.111	0.114	0.129	0.130													
Cneb	0.115	0.121	0.130	0.138	0.145	0.119	0.115	0.115	0.110	0.107	0.108	0.106	0.096	0.126	0.133	0.147	0.133	0.136												
Manc	0.174	0.179	0.184	0.176	0.180	0.176	0.175	0.176	0.181	0.169	0.181	0.176	0.174	0.174	0.171	0.185	0.177	0.177	0.185											
Mmor	0.182	0.183	0.193	0.189	0.189	0.179	0.182	0.183	0.185	0.175	0.187	0.188	0.181	0.175	0.174	0.191	0.183	0.186	0.184	0.061										
Irem	0.129	0.130	0.133	0.130	0.121	0.140	0.132	0.131	0.129	0.137	0.140	0.134	0.122	0.111	0.114	0.126	0.135	0.090	0.134	0.180	0.191									
Ialt	0.130	0.133	0.134	0.132	0.128	0.136	0.130	0.130	0.135	0.142	0.136	0.127	0.122	0.107	0.108	0.130	0.132	0.090	0.132	0.184	0.186	0.085								
Anob	0.122	0.129	0.127	0.129	0.132	0.120	0.125	0.124	0.119	0.121	0.118	0.111	0.110	0.118	0.124	0.138	0.096	0.129	0.120	0.179	0.192	0.134	0.132							
Spol	0.159	0.167	0.158	0.165	0.170	0.161	0.158	0.157	0.174	0.174	0.165	0.156	0.153	0.162	0.158	0.172	0.156	0.170	0.165	0.200	0.209	0.172	0.168	0.145						
Psqu	0.156	0.168	0.151	0.155	0.165	0.162	0.158	0.158	0.168	0.162	0.151	0.152	0.149	0.151	0.150	0.167	0.149	0.172	0.153	0.196	0.201	0.177	0.160	0.139	0.168					
Nocc	0.158	0.171	0.151	0.163	0.157	0.163	0.154	0.153	0.170	0.168	0.165	0.167	0.155	0.161	0.157	0.165	0.146	0.161	0.163	0.199	0.209	0.175	0.165	0.145	0.148	0.160				
Nmic	0.160	0.168	0.159	0.163	0.167	0.162	0.167	0.165	0.172	0.166	0.167	0.168	0.151	0.154	0.154	0.170	0.150	0.164	0.159	0.201	0.199	0.181	0.170	0.151	0.156	0.149	0.077			
Sill	0.190	0.204	0.191	0.194	0.204	0.194	0.190	0.189	0.197	0.201	0.193	0.207	0.195	0.192	0.196	0.210	0.193	0.201	0.213	0.223	0.234	0.210	0.200	0.186	0.206	0.196	0.187	0.187		
Barm	0.200	0.209	0.204	0.197	0.215	0.198	0.198	0.198	0.203	0.202	0.202	0.197	0.198	0.204	0.203	0.218	0.200	0.215	0.197	0.233	0.232	0.218	0.207	0.193	0.197	0.194	0.193	0.189	0.197	
Bron	0.208	0.213	0.211	0.210	0.223	0.215	0.204	0.206	0.209	0.210	0.218	0.214	0.205	0.212	0.210	0.230	0.208	0.218	0.220	0.233	0.245	0.225	0.215	0.203	0.196	0.200	0.199	0.202	0.200	0.092

New and Old Worlds. In our study, *A. nobilis* is closely related to the *Cynoscion* group and *C. guatucupa* is its sister taxon. The latter result was unexpected, especially since *C. guatucupa* occurs in the south Atlantic (Fig. 1) while *A. nobilis* occurs in the north Pacific, and suggests that *C. guatucupa* may belong in the genus *Atractoscion*. A formal taxonomic revision of this issue seems warranted. Finally, *Plagioscion* is a freshwater genus restricted to South America (Chao, 1978). It has been tentatively placed in the *Cynoscion* group by Chao based on its swim bladder, otoliths and external morphology, and by Sasaki (1989) based on having a pair of horn-like appendages extending from the swim bladder and reduced epipleaural ribs. Unfortunately, our data are ambiguous regarding its placement. *Plagioscion* falls out between the *Cynoscion* group and the other outgroup genera, and greater sampling of sciaenid genera is necessary to resolve its position.

Our analysis is the first comprehensive phylogeny for the *Cynoscion* group based on molecular data and provides a baseline for future comparative studies of this ecologically and economically important lineage. However, more work is needed to fully elucidate the evolutionary history of the group. A serious taxonomic revision to accompany our molecular analysis seems warranted given the lack of monophyly of genera in the group. Priorities for future research include greater taxon sampling to define the relationship between *Plagioscion* and other members of the *Cynoscion* group, collection of tissue samples from the five species of *Cynoscion* which we could not obtain, and sampling of nuclear genes.

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