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Research Article

Sciaenidae fish of the Caeté River estuary, Northern Brazil: mitochondrial DNA suggests explosive radiation for the Western Atlantic assemblage

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Abstract

Sciaenids are fish which are normally abundant in tropical estuaries of the western Atlantic. Studies on the Caeté river estuary in the northern Brazilian state of Pará have revealed that in this area Sciaenidae is the dominant family, comprising almost 50% of all teleosts sampled. In this paper we present the results of the first phylogenetic study on South American estuarine sciaenids, during which we obtained mitochondrial gene 16S sequences from 15 species belonging to eight genera occurring in the Caeté estuary. Intergeneric nucleotide divergences varied from 5 to 15%, *Lonchurus* and *Menticirrhus* being the most divergent lineages. Nucleotide divergences were quite variable amongst species of the same genus, ranging from 1.2% (*Stellifer microps* x *Stellifer naso*) to 8.4% (*Menticirrhus americanus* x *Menticirrhus littoralis*). Cladograms based on maximum parsimony, minimum evolution and maximum likelihood depicted an explosive diversification pattern for the western Atlantic sciaenid assemblage. Our analysis further reveals a very close relationship between *Bairdiella* and *Stellifer*, a monophyletic clade which emerged during the more recent diversification events of the Sciaenidae family. The phylogenetic reconstruction suggests the need for a revision of the taxonomy and nomenclature of the *Bairdiella*/*Stellifer* group.

Key words: Sciaenidae, Perciformes, mitochondrial DNA, rRNA 16S, Caeté River estuary.

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Introduction

Perciformes is the most varied of all orders of fish and the largest vertebrate group, containing 22 suborders, 150 families, some 1500 genera and approximately 9000 species (Nelson, 1994). Of this group, Sciaenidae is widely distributed throughout the world and is one of the largest families, with approximately 70 genera and 300 species, fish from this family being popularly known as croakers or drums due to the sound they produce using muscles associated with the swim bladder. They are mainly found in oceanic and estuarine waters, with only few genera (*Plagioscion*, *Pachypops* and *Pachyurus*) living solely in fresh water (Chao, 1978; Nelson, 1994). A complete list of Sciaenidae species may be found at the site http://www.fishbase.org/Nomenclature/NominalSpeciesList.cfm?family = Sciaenidae.

The greatest diversity of Sciaenidae species in the Western Atlantic occurs in tropical regions, with 18 genera (*Bairdiella, Ctenosciena*, *Cynoscion*, *Equetus*, *Isopisthus*, *Larimus*, *Lonchurus*, *Macrodon*, *Menticirrhus*, *Micropogonias*, *Nebris*, *Odontoscion*, *Ophioscion*, *Paralonchurus*, *Pogonias*, *Sciena*, *Stellifer* and *Umbrina*) and 42 species being reported off the coast of Venezuela (Cervigón, 1993). The remaining genera, except for *Sciena* and *Lonchurus*, occur along the coasts of southeastern and southern Brazil, albeit with only some 25 species (Menezes and Figueiredo, 1980).

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Sciaenids are normally abundant in western Atlantic tropical estuaries, studies undertaken in Tortuguero (Costa Rica), Orinoco (Venezuela), Sinnamary (French Guiana), Terminós Lagoon (Mexico) and Laguna Madre in the Gulf of Mexico show that Sciaenidae is one of the dominant families together with the families Ariidae, Carangidae, Clupeidae, Engraulidae, Gerreidae and Mugilidae (Blaber, 1997). Although ichthyic studies on species composition and dynamics in Brazilian estuaries are scarce, the Caeté river estuary in the northeastern Brazilian state of Pará has become the most studied area in Brazil over the last few years because of the Brazil/Germany bilateral scientific cooperation Mangrove Dynamics and Management (MADAM) project. Barletta (1999) evaluated the piscine species composition in different habitats of the Caeté estuary during September 96 to March 97, reported seven genera and 11 species of Sciaenidae (*Cynoscion acoupa, Cynoscion leiarchus, Cynoscion microlepdotus, Lonchurus lanceolatus, Macrodon ancylodon, Menticirrhus littoralis,*

Micropogonias furnieri, Plagioscion sp, Stellifer microps, Stellifer rastrifer and *Stellifer stellifer*). The structure and seasonal dynamics of larval fish in this estuary was also analyzed from July 1996 to September 1997 (Barletta-Bergan *et al*., 2002), and Sciaenidae was the most abundant family, comprising 46.5% (11 taxa) of all teleosts sampled. Except for members of the genera *Isopisthus*, both adult and larval sciaenid diversity were the same in both studies showing that Sciaenidae is a typical estuarine assemblage.

Envisaging the need for long-term studies on ichthyofauna dynamics in the Caeté and other northern Brazilian estuaries, our laboratory is selecting DNA molecular markers for identifying the most abundant species. The goal is to compare sequences of mitochondrial genes in larvae, juvenile and adult fish in order to enhance classification keys for species in the region and develop specie-specific DNA markers for phylogenetic systematics and population dynamics studies. The study reported in this paper is the first with a phylogenetic focus on South America estuarine sciaenids. Mitochondrial gene 16S sequences were obtained from 15 species of 8 genera from the Caeté river estuary, Bragança, Pará. The cladograms generated suggest a monophyletic origin and an explosive diversification for this group. Our analyses further reveals that *Bairdiella* and *Stellifer* are closely related and have appeared during the more recent diversification events, together with the appearance of the different *Stellifer* species. Phylogenetic reconstruction suggests the need for a revision of the taxonomy and nomenclature of this group.

Material and Methods

Fifteen species of Sciaenidae were caught using block nets at several sampling points (Furo do Taicy, Furo Grande, Praia de Ajuruteua, Vila de Ajuruteua and Ilha de Canelas) in the Caeté estuary (longitude 46°32'16" and 46°55'11" W and latitude 00°43'18" and 00°04'17" S) which is located some 150 km south of the mouth of the Amazon River in northeastern Brazil (Figure 1, Table 1).

Species were identified according to Chao (1978), Menezes and Figueiredo (1980) and Cervigón (1993). At least two individuals of each species were sequenced and no differences were found inside populations. Muscular tissue was extracted immediately from collected specimens and frozen or preserved in 95% ethanol at room temperature in the field then stored at 4 °C in the laboratory. To extract the DNA, tissues were digested for 1 h by ribonuclease at 37 °C, followed by a 2-4 h or overnight incubation at 55 °C with proteinase K. Total DNA was then purified by standard phenol/chloroform extraction followed by precipitation using isopropanol (Sambrook *et al*., 2001).

A 600 base pair fragment of the mitochondrial 16S rRNA gene was amplified using the primers originally described by Palumbi *et al.* (1991) with minor modifications to improve the amplification of material from Perciforme fish, the primers being 16S-L (5-CGCCTGTTTATCAA

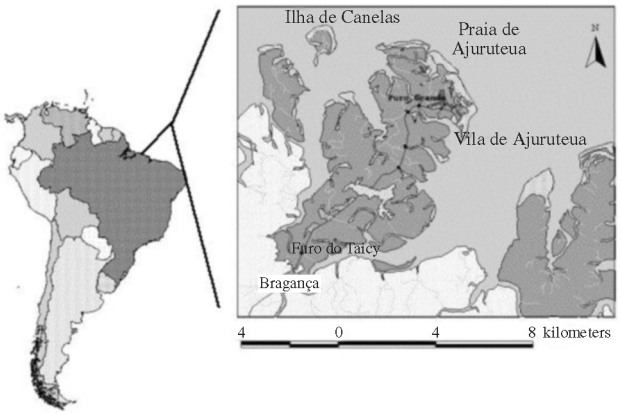

Figure 1 - Caeté river estuary in the northern Brazilian state of Pará, the research area of the Mangrove Dynamics and Management (MADAM) project. Samples of Sciaenidae were caught on Furo do Taicy, Furo Grande, Ilha de Canelas, Praia de Ajuruteua and Vila de Ajuruteua. This map was modified from Krause *et al*. (2001).

Table 1 - Rio Caeté estuary Sciaenidae species analyzed in the present study.

Species	Collection point
Bairdiella rhonchus	Ilha de Canelas
Cynoscion acoupa	Vila de Ajuruteua
Cynoscion leiarchus	Vila de Ajuruteua
Cynoscion microlepidotus	Vila de Ajuruteua
Cynoscion virescens	Vila de Ajuruteua
Lonchurus lanceolatus	Praia de Ajuruteua
Macrodon ancylodon	Praia de Ajuruteua
Menticirrhus americanus	Praia de Ajuruteua
Menticirrhus littoralis	Ilha de Canelas
Micropogonias furnieri	Ilha de Canelas
Nebris microps	Furo Grande
Stellifer microps	Furo do Taicy
Stellifer naso	Furo Grande
Stellifer rastrifer	Furo Grande
Stellifer stellifer	Furo do Taicy

AAACAT-3) and 16S-H (5-TTTCCCCGCGGTCGCCCC-3). Polymerase chain reaction (PCR) amplification was performed in 100 µL of reaction mixture containing 16 µL of dNTP (1,25 mM), 10 µL of buffer (10X conc.), 4 µL of $MgCl_2$ (25 mM), 1 µL of each primer (200 ng/µL), 5 µL of total DNA (200 ng/µL), 0.5 µL of Taq DNA polymerase (2U/µL) and 62.5 µL of pure water. A 2400 thermocycler (Perkin Elmer) was used for amplification, with a cycling profile of 94 °C for 3 min followed by 25 cycles of 94 °C (1 min)/50 °C (1 min)/72 °C (2 min), and 72 °C (10 min). Amplification products were purified using ExoSap IT (Amersham-Pharmacia) and submitted to a cycle-sequencing reaction using fluorescent-labeled di-deoxy ter-

minators supplied in the ABI PrismTM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). Sequencing reactions were performed in the 2400 thermocycler in 20 µL reaction mixtures containing 5 µL of DNA, 1 mM of primer, 2 µL of BigDye mix, 6 µL of buffer (200 mM Tris/5 mM MgCl2), with a profile of 96 °C (10 s)/50 °C (5 s)/60 °C (4 min). Unincorporated di-deoxynucleotides were removed by washing with isopropanol according to the method given in the ABI chemistry manual. Products were separated by electrophoresis at 3.000 V for 3 h and the sequences collected using the ABI Prism 377 automated sequencer (Applied Biosystems). Both strands of the 16S fragment were completely sequenced in all specimens. Sequences were deposited in GenBank under accession numbers AY603004-AY603018).

Edited sequences varying in size from 496 bp (*Nebris microps*) to 511 bp (*Menticirrhus americanus*) were aligned using the ClustalW (Thompson *et al*., 1994) implemented in BioEdit (Hall, 1999). Insertion/deletions (Indels) varying from one to 12 positions (bp) were inserted to improve the alignment. A region encompassing twelve nucleotide positions (sites 332 to 343) showing ambiguities on the alignment was excluded from the analyses. Gaps were considered as missing data on the phylogenetic reconstructions. Saturation of transitions in relation to transversions was evaluated using the data analysis in molecular biology and evolution (DAMBE) program (Xia and Xie, 2001).

Phylogenetic and molecular evolutionary analyses were conducted using the molecular evolutionary genetics analysis software version 2.0 (MEGA2; Kumar *et al*. 2001) and the phylogenetic analysis using parsimony (PAUP) program version 4.0b10 (Swofford, 2002). The Modeltest program (Posada and Crandall, 1998) was applied to estimate the model that best fited the 16S data. The model selected was the general time-reversible (GTR) model assuming a different rate for all six classes of substitutions with some sites assumed to be invariable and variable sites assumed to follow a gamma distribution (GTR+I+G). Robustness of the trees was evaluated using the Bremer decay index (Bremer, 1994) and 1000 bootstrap replicates for maximum parsimony and minimum evolution and 100 bootstrap replicates for maximum-likelihood (Felsenstein, 1985).

To test the monophyly of the Sciaenidae group from the Caeté estuary, phylogenetic trees were generated which included members of six other Perciformes families, the 16S sequences needed being retrieved from the GenBank for species of Nototheniidae (Richie *et al*; 1997), Cichlidae (Farias *et al*., 1999), Centropomidae (Tringali *et al*., 1999), Scaridae (Streelman *et al*., 2002); Sparidae (Hanel and Sturmbauer, 2000) and Serranidae (Craig *et al*., 2001). Alternate tree topologies were compared statistically using the topology-dependent tail permutation tests (T-PTP) with 1000 replicates (Archie, 1989; Faith, 1991).

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                                1111111111 1111112222
                         111225889 0011223346 6677790011
                        9489151286 0234390785 8901221802
Lonchurus lanceolatus   GCGAGCGACT AGAATAAGTA TAGCAACTAG
Macrodon ancylodon      T.AT.T.... ........CG ..AACGT..A
Micropogonias furnieri  T..T..A... ...G...... ..CT..TC.A
Menticirrhus americanus A..T..A.T. .CG.C.G... CCC.C.TC..
M. littoralis           ...T..A.AA .CG.C..... .CCTC.TC..
Nebris microps          T....TA... .......... .CCAC.TC..
Cynoscion acoupa        T.AG..AG.. .......... .TTAG.TC.A
C. virescens            T....TA... ...G.G..CG .TAA..TC.A
C. microlepdotus        T....TA... ...G..G... ACAAC.TC.A
C. leiarchus            T....TA... ...G..G.CG ACCG..TC.A
Bairdiella rhonchus     AT.TA.A... T.G....... .TAAC.A..A
Stellifer rastrifer     AT..A.A..A T......T.. CTTA..A..A
S. stellifer            AT..A.AG.A T......T.. .TAA..A...
S. microps              AT.TA.AGT. TA........ .TTA.GG.GA
S. naso                 AT.TA.AGT. TA...G.... .CTA.GG.GA

                        2222222222 2222222222 3333333333
                        1222233445 5666777999 0012233445
                        6367948675 8679089126 0160401681
Lonchurus lanceolatus   ATGATATTAA ATCTGCGCAG GCTCGATATC
Macrodon ancylodon      .CA.CG..G. G...AT.... A..A.C...T
Micropogonias furnieri  CCA.....GG G...AT.... AT...CC..T
Menticirrhus americanus GAAG.CAC.G ...C.T.... AA.AAC...T
M. littoralis           GCA..TAC.G T..C.T..G. ...AATAG.T
Nebris microps          .CA.CGC.GG GC..AT..G. AT...CC..T
Cynoscion acoupa        GCA.C.C.GG G...AT.... A.CA.CC..T
C. virescens            GC.GC...GG GC..AT.... A..A.CCG.T
C. microlepdotus        .CA.CCC.G. G...AT.... A..A.CCG.T
C. leiarchus            .CA.C...GG G...AT.... A..A..CG.T
Bairdiella rhonchus     ....G...G. GC...TATGA TTC..CA.CT
Stellifer rastrifer     .......C.. GC...TATG. AA.T.TA..T
S. stellifer            G.AG...C.. GC.GT.ATGA AT...TA..T
S. microps              ..A.C.C... GCT..TATG. .....TA.C.
S. naso                 ..A.C.CC.. GCT..TATG. .....TA.CT

                        3333333334 44444
                        5556667781 12356
                        3685697950 76545
Lonchurus lanceolatus   CCACTGGATC CATCC
Macrodon ancylodon      ..GT..A... T.C.T
Micropogonias furnieri  ...A..A... .C...
Menticirrhus americanus .A..AA..C. .G...
M. littoralis           .AGTCA.... TG...
Nebris microps          ......AGCT TG..T
Cynoscion acoupa        A.GT..A... .G...
C. virescens            ..GT..A... .G...
C. microlepdotus        ...TC.A.CT .G...
C. leiarchus            A.GTC.AG.. .G...
Bairdiella rhonchus     .TT..AA.CT TG...
Stellifer rastrifer     .TT...A... TG...
S. stellifer            .TT...A... T....
S. microps              .TT...A... T.CTT
S. naso                 .TT...A... T.CTT
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Figure 2 - Aligned DNA sequences of mtDNA 16S rRNA informative sites for Caeté estuary Sciaenidae.

Results

Nucleotide divergence and phylogenetic trees

The variable sites of the 16S alignment for the 15 sciaenid taxa are shown in Figure 2, the total length of the alignment being 517 sites with 376 invariants and 75 variable sites excluding deletions.

Intergeneric nucleotide divergences varied from between 4.8 to 15.3% (Table 2), with *Lonchurus* and *Menticirrhus* presenting the highest values in comparisons with the other Sciaenidae lineages. Divergence between species of the same genus was quite variable.

The minimum evolution, maximum likelihood and maximum parsimony phylogenetic trees show unresolved polytomy for the majority of Sciaenidae lineages, even for

species of the same genus as in the case of *Cynoscion* (Figure 3). Only a few clades were significantly supported by bootstrap values >90% and decay indexes higher than 5, e.g. *Bairdiella* x *Stellifer* (B = 98-99%, D = 9), *Stellifer microps* x *S. naso* (B = 99-100%, D = 13), *Stellifer rastrifer* x *S. stellifer* (B = 87-90%, D = 13) and *Menticirrhus americanus* x *M. littorallis* (98-99%).

Comparison of the sciaenids from the Caeté estuary with six other families of Perciformes produced significant bootstrap values (>90%), demonstrating the monophyly of the Caeté estuary sciaenids. Decay index analysis indicated that 11 extra mutations would be required to break up this arrangement (Figure 4). Although only one or two taxa from each of the other families are represented in the cladogram, full datasets were tested and always depicted the same topology and levels of support.

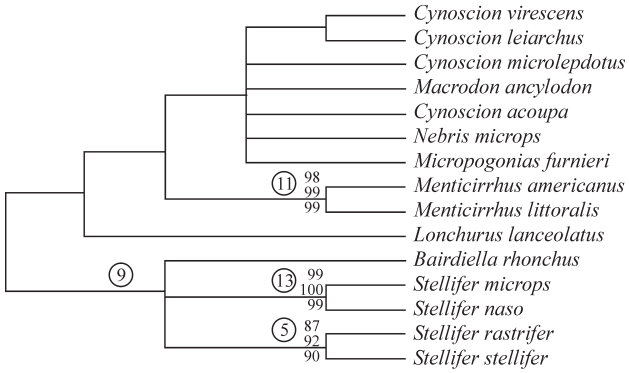

Figure 3 - Parsimony, minimum evolution and maximum likelihood consensus cladograms. A single MP tree (L = 287; CI = 0.684; rescaled CI = 0.395) was found. Numbers on the branches are ME bootstrap values obtained with 1000 replicates. Similar bootstrap values were obtained for the ML tree with 1000 replicates.

Discussion

The Sciaenidae family represents the most abundant piscine component of the Caeté River estuary. Eight genera and 11 species were described for the area in surveys undertaken in 1996 and 1997 (Barletta, 1999; Barletta-Bergan *et al.*, 2002), revealing the importance of this ecosystem on larval recruitment of this family. In our study we sampled a total of 8 genera and 15 species, representing the great majority of the Caeté estuary Sciaenidae assemblage.

The monophyly of the *Sciaenidae* group from the Caeté estuary

The phylogenetic cladogram using six other Perciforme families as outgroups (Figure 4) suggests that the Caeté estuary Sciaenidae assemblage constitutes a monophyletic clade. It should be noted, however, that this cannot be generalized to the Sciaenidae family as a whole or even to just the western Atlantic group because only 8 of the 70 genera of the family and of the 18 described by Cervigón (1993) as occurring in South American coastal waters were represented in our analysis. Nonetheless, western Atlantic sciaenids indeed appear to constitute an homogeneous group, quite different from the eastern Atlantic assemblage, which may indicate a single common origin for the Atlantic group. Eight genera (*Argyrosomus, Atractoscion, Miracorvina, Pentheroscion, Pseudolithus, Pteroscion, Sciena* and *Umbrina*) have been described for the eastern Atlantic (Quéro *et al*., 1990) and of these only

Table 2 - Percentage of nucleotide divergences of the mitochondrial 16S generated according to the general time-reversible (GTR) model, for 15 Sciaenidae species of the Caeté estuary river.

	Species													
Species	*Lla*	*Man*	*Mfu*	*Mam*	*Mli*	*Nmi*	*Cac*	*Cvi*	*Cmi*	*Cle*	*Brh*	*Sra*	*Sst*	*Smi*
Lonchurus lanceolatus (Lla)														
Macrodon ancylodon (Man)	12.0													
Micropogonis furnieri (Mfu)	8.7	9.1												
Menticirrhus americanus (Mam)	12.2	15.3	10.3											
M. littoralis (Mli)	12.4	13.9	9.8	8.4										
Nebris microps (Nmi)	10.1	8.7	5.5	10.3	10.0									
Cynoscion acoupa (Cac)	10.1	8.2	5.3	11.0	10.0	6.0								
C. virescens (Cvi)	9.6	7.5	4.8	11.0	10.0	5.5	4.2							
C. microlepdotus (Cmi)	10.5	8.2	5.9	10.1	10.3	4.6	5.5	4.4						
C. leiarchus (Cle)	10.1	8.4	5.3	11.0	9.8	5.7	5.1	3.1	3.7					
Bairdiella rhonchus (Brh)	10.8	11.7	8.6	12.2	11.7	7.3	8.9	8.4	8.9	10.5				
Steliffer rastrifer (Sra)	9.1	11.7	8.2	11.5	10.5	8.0	8.4	7.5	9.1	8.9	4.4			
S. stellifer (Sst)	9.8	12.9	9.1	12.6	11.4	8.9	9.6	8.7	11.0	11.0	5.7	3.3		
S. microps (Sm)	10.3	11.3	9.9	14.0	12.2	9.0	9.2	9.9	10.8	11.1	6.0	5.1	6.2	
S. naso	11.5	12.0	10.6	14.0	12.2	9.4	10.1	10.4	11.3	11.6	6.9	5.5	6.6	1.2

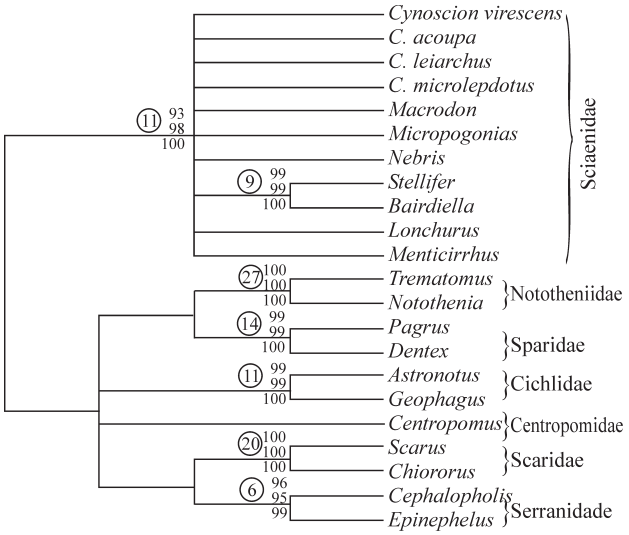

Figure 4 - Phylogenetic consensus tree for Caeté estuary Sciaenidae based on mtDNA 16S rRNA sequences. A single maximum parsimony (MP) tree (L = 623 and CI = 0.526) was found. Numbers on the branches are bootstrap values obtained with 1000 (MP at the top and minimum evolution (ME) in the middle) and 100 replicates (maximum-likelihood (ML) at the bottom). Only bootstrap values >90% are shown. Numbers inside the circles are Bremer decay indexes.

Sciaena and *Umbrina* (which were not represented in our study) have been reported for the western Atlantic (Menezes and Figueredo, 1980; Cervigón, 1993).

Intergeneric relationships

The cladograms generated from the 16S sequences present very small inter-nodes, indicating short intervals of time between the different diversification events of the western Atlantic Sciaenidae lineages. As can be seen in Table 2, intergeneric divergence varied from five to 15% but there was no evidence of saturation in the transition/transversion rate (graph not shown). This polytomic pattern for the western Atlantic sciaenid group was insufficient to elucidate clearly the phylogenetic relationships between the major lineages. Interestingly, analyses based on swim bladder, otolith or external morphology have presented similar difficulties in that such analyses have shown a polytomic arrangement for southwestern Atlantic sciaenids (Chao, 1978) with the majority of the genera studied being placed in a monotypic supra-genera category. Of the taxa studied by us, Chao (1978) places *Cynoscion* and *Macrodon* in the supra-genus *Cynoscion,* and *Stellifer* and *Bairdiella* in the supra-genus *Stellifer; Menticirrhus, Nebris* and *Micropogonias* are monotypic supra-genera, while *Lonchurus* is placed in the same supra-genus as *Paralonchurus* (not included in our analysis). Our data agree with Chao only in the grouping of *Stellifer* with *Bairdiella*. Although some of our cladograms present *Macrodon* near to *Cynoscion,* support for this link is not statistically significant.

Sasaki (1989) performed a similar morphological analyses which included all 70 genera of the Sciaenidae family but found strong support for only one group, *i.e. Bairdiella* and *Stellifer* (plus *Odontoscion*, *Elattarchus*, *Corvula* and *Ophioscion*). This group shares nine synapomorphies, the most important of which was the development of the swim bladder. The remaining groups are supported by only a single synapomorphy, those included in our analysis of western Atlantic genera being (i) *Micropogonias*, *Lonchurus* (and *Paralonchurus*) and (ii) *Nebris* (and *Larimus*), *Cynoscion*, *Macrodon*, *Plagioscion* (plus *Isopisthus* and five others from the eastern Pacific). Our analyses fully agree with the well-supported *Bairdiella*/*Stellifer* clade, but do not find any support for the weak single synapomorphy groups proposed by Sasaki (1989).

The close similarity between *Bairdiella* and *Stellifer*

Molecular data from our study revealed a marked similarity between *Bairdiella* and *Stellifer* species, supported by several synapomorphies. As can be seen in Figure 2, nine sites are made up of nucleotides shared exclusively by *Bairdiella* and *Stellifer*. For seven sites (14, 21, 100, 279, 291, 356 and 358) *Bairdiella* shares the derived nucleotide with all four species of *Stellifer*, while for two sites (201 and 296) *Bairdiella* shares the apomorphic character with at least one of the *Stellifer* species, although ambiguity or homoplasy was not observed in any of the sites in question. Besides these sites, site 9 may also be considered as supporting the existence of this clade because *Bairdiella* and *Stellifer* share one adenine that, although also present at the same site in *Menticirrhus littoralis,* is possibly the result of convergence. Analyzing the alternative arrangements for *Bairdiella* and *Stellifer* using maximum parsimony we found that only one site can support an alternative arrangement for *Bairdiella* (*i.e.* site 113 presents one exclusive guanine synapomorphy for *Bairdiella* and *Menticirrhus*) and two sites for *Stellifer* (*i.e.* site 246 groups *Stellifer* (without *S. microps*) with *Menticirrhus* because of a single shared cytosine and site 247 groups *Stellifer, Menticirrhus* and *Lonchurus* because of a shared adenine) (Figure 2). Further support for the *Bairdiella*/*Stellifer* group comes from the highly significant bootstrap values (>95%) for this clade (Figure 3), while the decay index (Bremer, 1994) for this node is 9 which means that nine additional mutations would be necessary to break this well-supported clade.

In the light of these results we must ask whether or not *Bairdiella* and *Stellifer* should be maintained in the currently accepted distinct taxonomic ranks or whether they are indeed different genera altogether. In order to resolve this question the arrangement among the different *Stellifer* species and between these and *Bairdiella* must be elucidated. The distance matrix (Table 2) and the parsimony, minimum evolution and maximum likelihood consensus cladograms (Figure 3) show that the four Caeté estuary

Stellifer species constitute two distinct and possibly monophyletic groups, one including *S. microps* and *S. naso* (which have only 1.2% nucleotide divergence and a bootstrap value of 100%) and the other *S. stellifer* and *S. rastrifer* (3.3% nucleotide divergence and a bootstrap value of 85%). The emergence of *Bairdiella* seems to have occurred very close to the first diversification events in *Stellifer* as may be inferred from the nucleotide divergence values which are 5.1 to 6.6% between the two groups of *Stellifer* and 4.4 to 6.9% between *Bairdiella* and the *Stellifer* species. Moreover, the node that joins both *Stellifer* groups presents a non-significant bootstrap value of less than 50% (Figure 3), indicating that *Bairdiella* is in fact closely linked to *Stellifer*. Based on this similarity, the t-PTP test (Archie 1989; Faith 1991) significantly rejects ($p = 0.001$) the monophyly of *Stellifer* without having *Bairdiella* included in the grouping, indicating that a polytomy best represents the relationship of *Bairdiella* and the two groups of *Stellifer* species. Consequently, to be congruent with the phylogenetic reconstruction, we suggest that *Bairdiella* and *Stellifer* should be considered as belonging to the same taxonomic rank. Our results are in agreement with previous morphology-based studies that suggest a high degree of similarity between *Bairdiella* and *Stellifer* (Chao, 1978; Sasaki, 1989).

Evolutionary reconstruction from molecular data indicates the occurrence of at least three bouts of diversification for the *Bairdiella*/*Stellifer* group: the first bout isolated the ancestors of what is now *Bairdiella*, the proto-*microps*/*naso* and proto-*stellifer*/*rastrifer*, the second gave rise to *Stellifer rastrifer* and *S. stellifer*, and the third and most recent resulted in *S. microps* and *S. naso*.

The evidence accumulated in the classical morphology-based studies and phylogenetic reconstruction from the molecular data described in this paper indicates that *Bairdiella* and *Stellifer* should be included in a single taxonomic rank and since *Bairdiella* Linnaeus (1758) was described before *Stellifer* Block (1790), *Bairdiella* should be given priority in the redefinition of the nomenclature. Obviously, such a dramatic change in the taxonomy must be preceded by a study including the 21 *Stellifer* and 7 *Bairdiella* species described in the FishBase (http://www.fishbase.org/Nomenclature/NominalSpeciesList.cfm?family = Sciaenidae).

High nucleotide divergence between *Menticirrhus* species

Another surprising finding which came to light during our study was the high degree of nucleotide divergence (8.3%) observed between the two Caeté estuary *Menticirrhus* species, the highest observed divergence between species of the same genus, which was even greater than some of the intergeneric divergence values occurring within the Sciaenidae family. In order to better understand the nucleotide divergence patterns and age of the species within this genus more detailed analysis needs to be undertaken which includes all of the 9 *Menticirrhus* species distributed in the Atlantic and Pacific.

Explosive diversification in *Cynoscion*

Four *Cynoscion* species were included in our analysis, the nucleotide divergence between them ranging from 3 to 6% (Table 2). The polytomic topology (Figures. 3 and 4) and low bootstrap values do not support monophyly of the genus *Cynoscion*. As can be seen in Table 2, species of *Cynoscion* present low divergence values in relation to species from other genera, such as *Micropogonias, Nebris* and *Macrodon*. Only if we exclude *Micropogonias*, *Macrodon* and *Nebris* from the analysis can we generate a cladogram with a bootstrap value of 99% supporting the monophyly of *Cynoscion,* strengthening the hypothesis of explosive radiation in this genus. Nonetheless, because *Cynoscion* is the genus with the highest number of species (25 described in the FishBase) within the family Sciaenidae and the widest geographical distribution, broader studies are necessary to verify whether or not this genus is monophyletic.

Final comments

Our phylogenetic analysis based on 16S mitochondrial gene DNA sequences revealed that explosive radiation has occurred in the extant western Atlantic sciaenid genera. Most of the eight genera represented in our analyses appear as a polytomic and unresolved arrangements in our phylogenetic trees. Even the monophyly of *Cynoscion*, represented in our analysis by four species, could not be demonstrated, suggesting a similar diversification pattern for this genus.

A significantly supported clade demonstrates a very close relationship between *Bairdiella* and *Stellifer* which, taking into consideration that close similarity has also been demonstrated by morphological arrangements, indicates a need for revision of the taxonomy of this group.

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