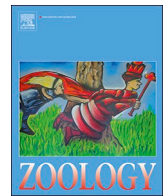




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Phylogenetic relationships of the species of *Plagioscion* Gill, 1861 (Eupercaria, Sciaenidae)

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ABSTRACT

The genus *Plagioscion* Gill, 1861 (Sciaenidae; Eupercaria) is currently composed of five valid species (*P. squamosissimus*, *P. auratus*, *P. magdalenae*, *P. ternetzi* and *P. montei*) widely distributed in South America. This study seeks to test the monophyly of *Plagioscion* and develop an hypothesis of phylogenetic relationships among the species of the genus, based on the analysis of 28 characters of external and internal morphology and behavior. The ingroup consists of the five species of *Plagioscion*. The outgroup was composed of eight species of the genera *Ctenosciaena*, *Cynoscion*, *Isopisthus*, *Larimus*, *Macrodon* and *Pachyurus*. Cladistic analysis yielded a single most parsimonious cladogram (L = 50, CI = 0.60 and RI = 0.72). The monophyly of genus *Plagioscion* was supported by the presence of five synapomorphies, corroborating previous studies. The single most parsimonious cladogram recovered the following hypothesis of relationships among the species of the genus: ((*P. squamosissimus* + *P. ternetzi*) (*P. magdalenae* (*P. montei* + *P. magdalenae*))). Comments about all characters are presented.

1. Introduction

Plagioscion Gill, 1861 (Sciaenidae) is a genus of Neotropical freshwater fishes widely distributed in South America, from Venezuela to Paraguay, in the Río Orinoco, Río Magdalena, Río Amazonas, Río Tocantins, Río São Francisco, Río Paraguay, and Río Paraná basins, and in rivers of the Guianas (Casatti, 2000 and 2005). Species of *Plagioscion* are popularly known as "pescadas", "pescada-branca", "pescada-amarela", or "corvina" and are very important for commercial fisheries (Hahn et al., 1999), reaching up to 80 cm total length (e.g., *P. squamosissimus*; Casatti, 2003). It is particularly abundant in the Amazon basin, where it is a popular market fish (Santos et al., 2006).

This genus is easily identified by its peculiar lateral-line scaling, which extends to the caudal fin. Species of *Plagioscion* are characterized by variations in teeth size and in the second anal-fin spine length (Campos, 1942). Sasaki (1989) proposed two synapomorphies for *Plagioscion*: (1) intercalar of auditory bulla projecting downward and (2) modified lateral-line scales, formed by smaller scales covering larger scales.

Species of *Plagioscion* have been described and are diagnosed based on morphometric and meristic features; however, they are not easily identified owing to the high variation among populations (Casatti, 2005), which resulted in descriptions of species that were later

regarded as synonyms. One of the most important characters used in species diagnosis is the second anal-fin spine; however, this character may be highly variable in *P. squamosissimus*, and may extend beyond the limit proposed for its congeners (Casatti, 2005). The genus *Plagioscion* is currently comprised of five valid species: *Plagioscion squamosissimus* (Heckel, 1840), widely distributed in drainages of the Atlantic Ocean, east of the Andes; *P. auratus* (Castelnau, 1855) in Río Orinoco and Río Amazonas basins; *P. magdalenae* (Steindachner, 1878) in Río Magdalena and Río Amazonas basins; *P. ternetzi* Boulenger, 1895 in the lower Río Paraná, Río Paraguay, and Río Uruguay basins; and *P. montei* Soares and Casatti, 2000 in the Río Amazonas basin.

The first contribution, adopting a cladistic paradigm in relation to *Plagioscion*, was proposed by Sasaki (1989), who analyzed swim-bladder, otoliths, and osteological and myological characters. Sasaki (1989) proposed that Sciaenidae was a natural group and contributed to the understanding of phylogenetic relationships and classification of several genera. However, not all *Plagioscion* species were analyzed, and the relationship between species remains unknown. According to Sasaki (1989), *Plagioscion* is related to marine genera *Cynoscion*, *Macrodon*, and *Isopisthus*, and their synapomorphies are few and reduced epipleural ribs. Subsequently, Casatti (2000, 2001, 2002a,b, 2005) contributed with a better understanding of phylogenetic relationships and taxonomy of South American freshwater drums of the genera

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Plagioscion, *Pachyurus*, and *Pachypops*. More recently, Cooke et al. (2011) and Lo et al. (2015) proposed phylogenetic relationships based on molecular data, and obtained relatively distinct topologies. Despite recent efforts, there is no consensus as to the phylogenetic position of *Plagioscion*. Casatti (2000) considered *Plagioscion* to be more related to *Otolithoides* and *Panna* (Indo-Pacific), whereas Boeger and Krisky (2003) suggested a relationship between *Plagioscion* and *Paralonchurus* based on the phylogeny of Dactylogyridae parasites.

The aim of the present paper is to test the monophyly of this genus and propose phylogenetic relationships among the five *Plagioscion* species based on morphological characters.

2. Material and Methods

2.1. Osteology and illustrations

The protocol by Taylor and Van Dyke (1985) was used for the osteological analysis and dissection was performed according to Weitzman (1974). Otoliths were extracted and prepared according to Secor et al. (1991), and cleaned by immersion in diluted sodium hypochlorite and distilled water to remove membranes. The terminology used for otolith morphology and structures follows Hecht (1987), Bastos (1990) and Corrêa and Vianna (1992). Osteological nomenclature followed Topp and Cole (1968), Taniguchi (1969) and Sasaki (1989). Osteological illustrations were prepared with the help of a stereomicroscope with a clear chamber. Some characters and respective states were illustrated using digital photography from an automated stereomicroscope.

2.2. Phylogenetic analysis

To hypothesize phylogenetic relationships, external morphological features (morphometric and tegument), osteological characters, otoliths, and swimbladder were analyzed through direct observation of cleared and stained specimens or of specimens compiled from previously published studies (Sasaki, 1989; Casatti, 2000, 2001, 2002a, 2005). Phylogenetic relationships followed the cladistic approach formulated by Hennig (1966) and subsequently improved by authors such as Wiley (1981), Nixon and Carpenter (1993), and Forey et al. (1994). Polarity of the characters analyzed was established using information examined in outgroups (Nixon and Carpenter, 1993). To construct the character matrix, the primitive state (plesiomorphic) was coded as "0" and the derived state (apomorphic) was coded as "1"; characters not susceptible to comparisons or undetermined were coded as "-". Most characters (26 out of the 28) were treated as binary, except for characters 12 and 17, which were treated as multi-state; character 12 was additive and character 17 was non-additive.

The character matrix was constructed using the Mesquite 2.7 program (Maddison and Maddison, 2009). Data matrices for phylogenetic analyses were assembled with the program Mesquite ver. 3.10. Parsimony analysis was implemented with the program TNT ver. 1.5 (Tree analysis using New Technology, Goloboff and Catalano, 2016; freely available through the Willi Hennig Society). Calculations used traditional searches, 10 random seeds, 1000 replicates, TBR algorithm with 1000 replicates, and retaining 2000 trees per replicate using a MultTBR + TBR search strategy. Winclada 1.00.08 was used to view synapomorphies, transitions between character states, and to identify consistency and retention indices in the tree and characters. Simultaneously, an analysis was also performed using Paup 4.0b10 (Swofford, 2003), Apple Macintosh version, using the exact search mechanism of "Branch and Bound" and viewing the most parsimonious tree in MacClade 4.0.3 (Maddison and Maddison, 2000). Bremer's decay index (Bremer, 1994) was calculated using TNT 1.0 (Goloboff et al., 2005) and TreeRot version 2 (Sorenson, 1999). The five valid *Plagioscion* species were used for the ingroup. Species were selected as outgroups according to the hypothesis of phylogenetic relationship of Sciaenidae

by Sasaki (1989), Cooke et al. (2011), and Lo et al. (2015), regarding the genera *Cynoscion*, *Macrodon*, and *Isopisthus* (*Cynoscionini* tribe) as primary outgroups. Other Sciaenidae taxa were added to the analysis to expand the outgroup, and were treated as a secondary outgroup: *Ctenosciaena gracilicirrhus* (Sciaeninae), *Larimus breviceps* (Lariminae), *Pachyurus schomburgkii* (Pachyurinae). Rooting was performed *a posteriori* to determine the direction of characters (Nixon and Carpenter, 1993) in the clade containing the species *Ctenosciaena gracilicirrhus*, basic Sciaenidae taxon (Sasaki, 1989). The description of characters is shown in the same order as the matrix (anatomical sequence rostral to caudal) followed by coding of states, consistency indices (ci), and retention indices (ri) according to Farris (1989), number of steps, taxa with the respective apomorphic states (in the matrix order), description, and discussion on the characters and their distribution among the species analyzed.

2.3. Institutional abbreviations

Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá, Macapá (IEPA); Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP); Museu Paraense Emílio Goeldi, Belém (MPEG); Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP).

2.4. Material examined

In group: *Plagioscion auratus*; MPEG 3463, 5, 135.96–188.89 mm SL; MPEG 9868, 9, 134.38–266.42 mm SL. *P. squamosissimus*; MPEG 8479, 5, 102.73–148.91 mm SL; MPEG 8456, 2, 183.42–218.27 mm SL; MPEG 1667, 1, 146.26 mm SL; MPEG 3594, 1, 133.54 mm SL; MPEG 2947, 4, 107.87–135.64 mm SL; MPEG 3594, 2, 131.23–167.02 mm SL; MPEG 4322, 6, 164.23–185.70 mm SL; MPEG 2947, 4, 107.87–135.64 mm SL; *P. magdalenae*; MPEG 2589, 2, 153.16–194.23 mm SL; MPEG 5191, 1, 136.91 mm SL; MPEG 2794, 2, 148.47–155.26 mm SL; MZUSP 34077, 4, 126.9–147.78 mm SL; MPEG 2795, 4, 108.61–108.84 mm SL; *P. montei*; MPEG 808, 2, 83.44–146 mm SL; MPEG 1671, 1, 107.64 mm SL; MZUSP 45954, 4, 124.5–216.2 mm SL; MPEG 1663, 2, 107.64–173.83 mm SL; MCP 32751, 1, 171.17 mm SL; *P. ternetzi*; MZUSP 21145, 5, 162.68–357.0 mm SL. Out group: *Cynoscioninae*: *Cynoscion acoupa*; MZUSP 68719, 3, 134.91–138 mm SL; IEPA 576, 1, 189.58 mm SL. *C. jamaicensis*; MCT 5170, 1, 91.4 mm SL; MCT 3536, 1, 107.58 mm SL; MCT 5174, 1, 95.24 mm SL; MCT 3536, 1, 119.34 mm SL; MCT 5171, 1, 80.08 mm SL; MCT 5169, 1, 85.890 mm SL. *C. leiarchus*; MZUSP 68857, 5, 36.10–113.9 mm SL. *C. striatus*; MCT 7128, 1, 93.28 mm SL; MCT 7177, 1, 97.89 mm SL. *Isopisthus parvipinnis*; MPEG 3473, 4, 101.89–134.33 mm SL; MCT 5193, 1, 83.57 mm SL; MCT 5193, 1, 84.30 mm SL; MCT 7317, 1, 102.01 mm SL; MCT 6484, 1, 91.2 mm SL; MCT 7316, 1, 109.58 mm SL; MPEG 3471, 6, 71.21–168.08 mm SL. *Macrodon ancylodon*; MPEG 3521, 2, 133.65–179.68 mm SL; MCT 3315, 1, 140.7 mm SL; MCT 5537, 1, 142.54 mm SL; MCT 3361, 1, 73.11 mm SL; MCT 3315, 1, 145.23 mm SL; MCT 5537, 1, 144.67 mm SL; MCT 3381, 1, 68.54 mm SL. *Lariminae*: *Larimus breviceps*; MZUSP 69139, 4, 70.81–108.51 mm SL. *Pachyurinae*: *Pachyurus schomburgkii*; MPEG 807, 3, 122.7–145.84 mm SL; MPEG 815, 5, 123.40–148.97 mm SL. *Sciaeninae*: *Ctenosciaena gracilicirrhus*; MZUSP 68699, 5, 70.14–88.82 mm SL; *Umbrina canosai*; MZUSP 70517, 5, 84.4–92.42 mm SL.

3. Results and Discussion

A total of 28 characters was obtained (Table 1), encompassing external morphology (tegument and morphometry), otolith morphology, swimbladder morphology, osteology, and freshwater habit. Osteological characters refer to neurocranium, vertebrae, caudal skeleton, pectoral, pelvic, and anal fins. A single cladogram was obtained when analyzing parsimony, with 50 steps, CI = 0.60, and RI = 0.72.

Table 1

Matrix of characters for phylogenetic analysis of *Plagioscion* species based on morphological, morphometric and meristic data. Hyphen (-) represents not applicable state. Asterisk (*) stands for rooting taxa.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
<i>Ctenosciaena gracilicirrhus*</i>	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0	0	0
<i>Cynoscion acoupa</i>	0	0	-	0	0	1	0	0	1	1	0	2	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0
<i>Cynoscion jamaicensis</i>	0	0	-	0	0	1	0	0	1	1	0	2	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0
<i>Cynoscion leiarchus</i>	0	0	-	0	0	1	0	0	1	1	0	2	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0
<i>Isopisthus parvipinnis</i>	0	0	-	0	0	1	0	0	1	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0
<i>Larimus breviceps</i>	0	1	0	0	0	1	1	1	0	0	0	0	-	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0
<i>Macrodon ancylodon</i>	0	0	-	0	0	1	0	0	1	1	1	2	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0
<i>Pachyurus schomburgkii</i>	0	0	-	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>Plagioscion auratus</i>	0	1	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1
<i>Plagioscion magdalenae</i>	1	1	0	1	1	0	1	1	0	0	0	1	0	1	1	0	1	0	0	1	1	1	1	1	1	0	1	0
<i>Plagioscion montei</i>	1	1	1	1	0	0	1	0	0	0	0	1	0	1	1	0	1	0	0	1	1	1	1	1	0	1	1	1
<i>Plagioscion squamosissimus</i>	1	1	1	1	0	1	0	0	1	1	1	1	0	0	0	0	2	1	0	1	1	1	1	1	1	0	0	1
<i>Plagioscion ternetzi</i>	0	1	1	1	0	1	0	0	1	0	1	1	0	0	0	0	2	1	0	1	0	1	1	1	1	0	0	1

Out of the 28 characters analyzed, 18 characters are original and ten were obtained from the literature (Sasaki, 1989; Casatti, 2000, 2001, 2002a, b; and 2005).

3.1. Characters

3.1.1. Neurocranium

[1] Relationship between basisphenoid and parasphenoid: (0) joined by bone projection; (1) separated; [ci = 0.50; ri = 0.50; Steps = 2].

Plagioscion magdalenae, *P. montei*, and *P. squamosissimus*

Basisphenoid and parasphenoid contribute to the formation of the interorbital septum together with lateral ethmoids. The basisphenoid is a unique "Y"-shaped bone and it is compressed laterally (Topp and Cole, 1968; Sasaki, 1989; Shinohara, 1994). It is generally connected to the pterosphenooid, prootics, and it is linked through its central portion by bone expansions to the dorsal keel of the posterodorsal section of the parasphenoid, which is long, and extended below the orbit, joining otic and olfactory regions at the basal part of the cranium (Topp and Cole, 1968; Sasaki, 1989). Sasaki (1989) mentioned that the basisphenoid is linked to the dorsal keel of the parasphenoid in most species of Sciaenidae and percoids, and considers this the plesiomorphic condition for the family (Fig. 1A). However, Sasaki (1989) mentioned that some species have the basisphenoid separated from the dorsal region of the parasphenoid (Fig. 1B), considering it as an apomorphy shared by *Paralanchurus brasiliensis*, *Pseudotolithus* and *Plagioscion auratus*, but observing the plesiomorphic state in *Plagioscion ternetzi*. Unlike Sasaki (1989), Casatti (2000) observed a plesiomorphic condition of the character in *Plagioscion auratus*. Here, as well as in the previously mentioned studies, the character condition represented by the basisphenoid linked to the parasphenoid through a bone projection was observed in all outgroup species, and is considered to be plesiomorphic. In the ingroup analysis, *P. auratus* and *P. ternetzi* had the plesiomorphic condition for the character, as observed by Casatti (2000). Thus, the apomorphic state is a synapomorphy for *P. magdalenae* and *P. montei*, and homoplastic in *P. squamosissimus*.

[2] Dorsal projection of parasphenoid: (0) absent; (1) present; [ci = 0.50; ri = 0.80; Steps = 2].

Larimus breviceps, *Plagioscion auratus*, *P. magdalenae*, *P. montei*, *P. squamosissimus*, and *P. ternetzi*

The parasphenoid is a unique, long bone, situated at the basal part of the cranium, uniting the optic and olfactory regions, generally linking the posterodorsal region to the basisphenoid (see character 1). At a ventral view, its posterior portion is linked to the prootics and basioccipital [Weitzman, 1962 (60, Fig. 4); Topp and Cole, 1968 (910, Fig. 2)]. It has a dorsal keel, generally smooth (Topp and Cole, 1968). Casatti (2000) regarded the smooth dorsal keel with no projections as plesiomorphic. The apomorphic state presents the parasphenoid with a

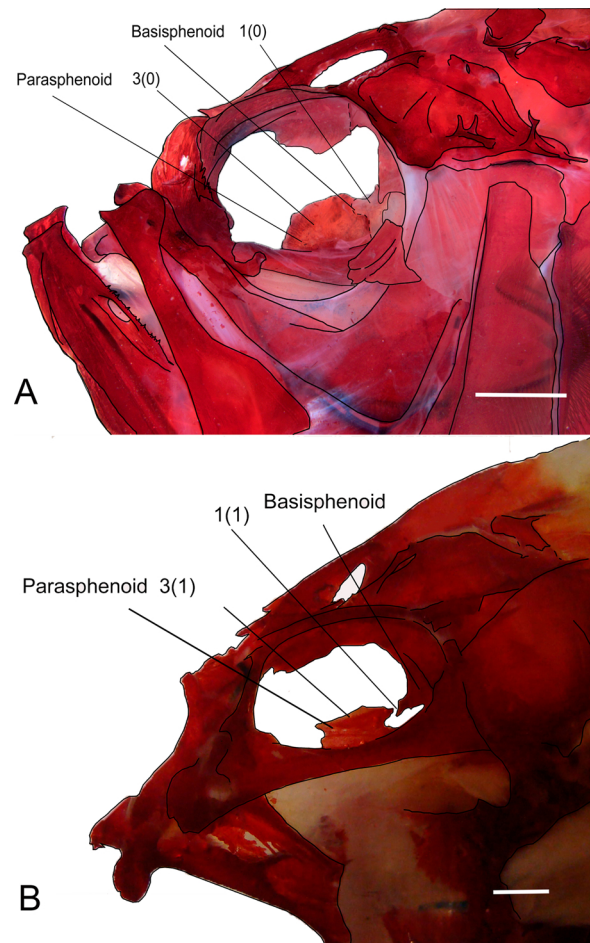


Fig. 1. Ocular orbit in left lateral view of: A) *Larimus breviceps*, MZUSP 69139, 86.33 mm SL; B) *Plagioscion squamosissimus*, MPEG 8479, 148.71 mm SL. Bar = 2 mm.

dorsal projection as a synapomorphy for the genus *Plagioscion*, with a reversal in *P. magdalenae*, which has parasphenoid without projection.

In the present study, the plesiomorphic state, represented by the dorsal portion of the parasphenoid smooth (Fig. 2A), was observed to be widely distributed in the outgroup, except in *Larimus breviceps*; in contrast to Casatti (2000), the presence of a dorsal projection was observed in all the specimens of *Plagioscion*. However, it is worthy of note that two different shapes of this parasphenoid projection were observed, which are analyzed in character 3. The apomorphic state (Fig. 2B) is shared by outgroup species *P. squamosissimus*, *P. auratus*, *P.*

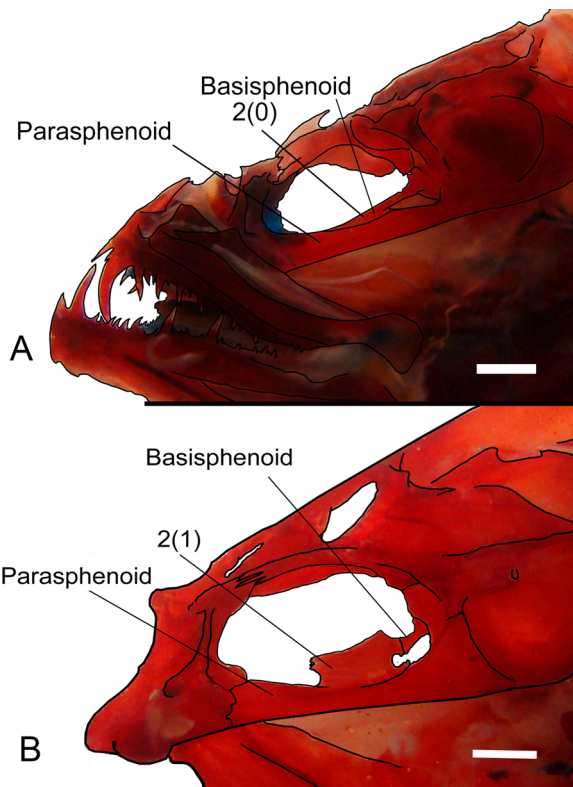


Fig. 2. Ocular orbit in left lateral view of: A) *Macrodon ancylodon*, MPEG 3521, 133.65 mm SL; B) *Plagioscion ternetzi*, MZUSP 21145, 155.04 mm SL. Bar = 2 mm.

magdalenae, *P. montei*, *P. ternetzi*, and by *L. breviceps*. However, in parsimony analysis, the presence of the dorsal projection of the parasphenoid represents a synapomorphy for a larger group involving *Larimus* and *Plagioscion*, with a reversal in the sister clade of *Plagioscion*, comprised of *Isopisthus*, *Cynoscion*, and *Macrodon*.

[3] Shape of dorsal projection of parasphenoid: (0) with circular rim; (1) dagger-shaped [ci = 0.33; ri = 0.50; Steps = 2].

Plagioscion montei, *P. squamosissimus*, and *P. ternetzi*

As described in character 2, the plesiomorphic condition of the parasphenoid with a smooth dorsal portion is widely distributed in the sciaenids and in percoids (Sasaki, 1989). The parasphenoid with a dorsal projection represents a derived condition. However, among *Plagioscion* species, two states regarding the shape of this dorsal projection have been observed. *Plagioscion auratus* and *P. magdalenae* showed dorsal projection with a thin, visually translucent circular rim, a condition which is also found in *L. breviceps* (Fig. 1A). In *P. squamosissimus*, *P. montei*, and *P. ternetzi*, this dorsal projection is dagger-shaped and thick (Fig. 1B). As a form of character polarity, the parasphenoid projection with a circular rim was considered to be plesiomorphic as it is shared with the outgroup. The apomorphic state represents a synapomorphy for *Plagioscion squamosissimus* and *P. ternetzi*, and is a homoplasy in *P. montei*.

[4] Intercalar: (0) without ventral projection; (1) with ventral projection, forming part of the auditory bulla (Fig. 3); [ci = 1.0; ri = 1.0; Steps = 1].

Plagioscion auratus, *P. magdalenae*, *P. montei*, *P. squamosissimus*, and *P. ternetzi*

The intercalar occurs in the posterior part of the cranium, typically in contact anteriorly with the prootic, dorsally with the pterotic, and ventrally with the exoccipital (Sasaki, 1989; Shinohara, 1994). In the genus *Plagioscion*, the intercalar projects downward, forming a large portion of the auditory bulla, as observed by Sasaki (1989) and confirmed in the present paper. The auditory bulla is rounded and

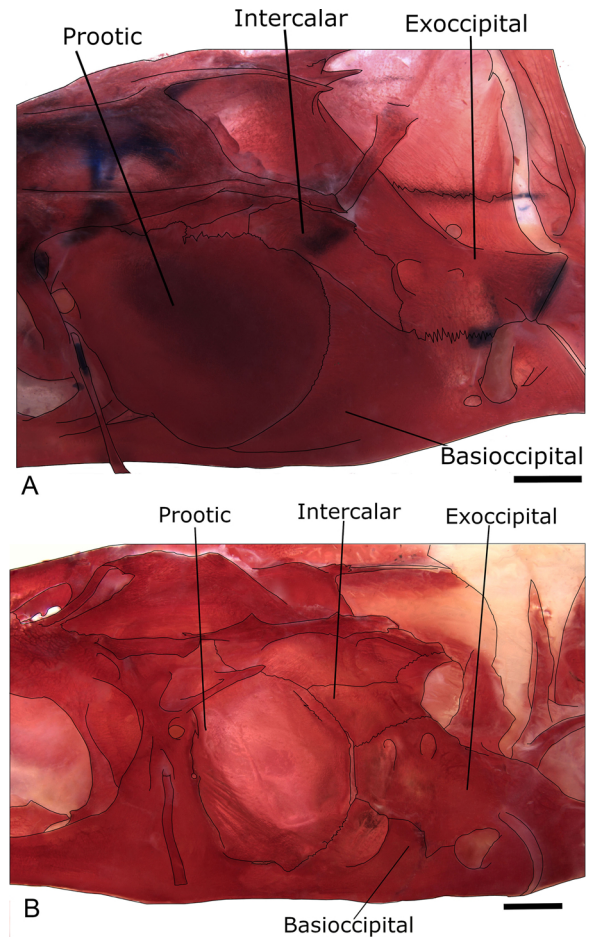


Fig. 3. Neurocranium in left lateral view of: A) *Macrodon ancylodon*, MPEG 3521, 133.65 mm SL; B) *Plagioscion squamosissimus*, MPEG 8479, 148.71 mm SL. Bar = 2 mm.

expanded, containing large otoliths (*Sagitta*), and is also formed by the prootic and basioccipital. The shape of the intercalar projecting downward was proposed by Sasaki (1989) as a synapomorphy for the genus *Plagioscion*, although it occurs as a homoplasy in species of other genera. Sasaki (1989) regarded the intercalar not being part of the auditory bulla as plesiomorphic (Fig. 3A), a condition broadly distributed in the species of Sciaenidae and in other percoids. Casatti (2000) used the states and the polarity in Sasaki (1989); however, Casatti (2000) did not obtain the same result, as the apomorphic state unites other genera to *Plagioscion*. In the present paper, only *Plagioscion* species presented the apomorphic state in this character (Fig. 3B), which reinforces that the intercalar as part of the auditory bulla is a synapomorphy for *Plagioscion*.

[5] Width of the frontal in the interorbital region: (0) wide, more than 21% of the length of the head; (1) narrow, up to 17% of the length of the head [ci = 1.0; ri = 0; Steps = 1].

Plagioscion magdalenae

The frontal is the largest paired bone, forming most of the anterior half of the dorsal skull roof, in contact with the supraoccipital, parietal, and the pterotic posteriorly, with the ethmoid and lateral ethmoid anteriorly, and with the sphenotic and pterosphenoid posteroventrally. The frontal is highly cavernous, with a series of ridges, a common characteristic of the Sciaenidae owing to the hypertrophy of the cephalic lateral line system (Sasaki, 1989).

The width of the frontal in the interorbital region was a character detailed by Casatti (2000), who recognized *Plagioscion magdalenae* and *Pachyurus squamipinnis* as species with a smaller width in the interorbital region, resulting in a very narrow frontal with a strong

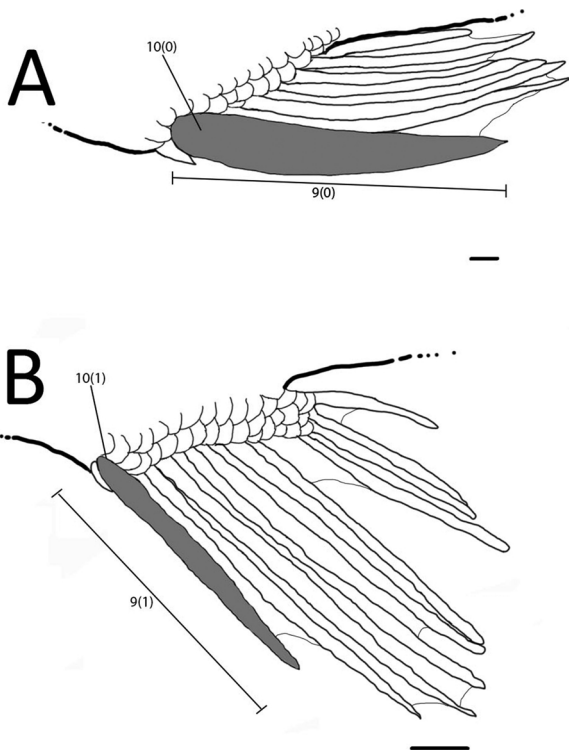


Fig. 4. Left lateral view of anal fin of: A) *Plagioscion auratus*, MPEG 3463, 188.89 mm SL; B) *Plagioscion squamosissimus*, MPEG 2947, 135.40 mm SL. Bar = 2 mm. In gray the second anal-fin spine; characters 9 and 10, in parentheses the respective states.

depression parallel to the horizontal through eye.

The plesiomorphic state was identified in the outgroup as having a relatively wider frontal, more than 21% of the length of the head. The apomorphic state was standardized with its interorbital distance up to 17% of the length of the head. In the present paper, only *Plagioscion magdalenae* had the apomorphic state represented by the smaller width of the frontal in the interorbital region as a consequence of a narrow frontal. Although the apomorphic state represents an autapomorphy of *P. magdalenae*, this character was chosen for its taxonomic value, frequently used to identify this species (Campos, 1942; Ferreira et al., 1998; Keith et al., 2000; Casatti, 2005), which is recognized as having the most narrow interorbital width, due to a very narrow frontal, and not having a morphometric overlap with its congeners, recognized as a satisfactory diagnostic character to discriminate it among the other species (Casatti, 2005).

[6] Distance from the posterior tip of the supraoccipital to the insertion of the first dorsal-fin spine: (0) up to 8% of the standard length; (1) 11% of the standard length or higher [ci = 0.50; ri = 0.75; Steps = 2].

Cynoscion acoupa, *C. jamaicensis*, *C. leiarchus*, *I. parvipinnis*, *Larimus breviceps*, *M. ancylodon*, *Plagioscion squamosissimus*, and *P. ternetzi*

Specimens of *Plagioscion squamosissimus* and *P. ternetzi* have the dorsal-fin origin relatively more posterior than *P. auratus*, *P. magdalenae*, and *P. montei*. We decided not to consider the pre-dorsal length (from the snout to the dorsal-fin origin), as the species have distinct proportions of length of the head, thus hampering the definition of the interval that would discriminate this character. Consequently, the distance from the posterior tip of the supraoccipital to the base of the first dorsal-fin spine was considered. This character was observed in cleared and stained specimens; thus it is possible to view and measure the distance between components without compromising the position of the fin in the specimen. The plesiomorphic state was identified as a short distance, defined as up to 8% of the standard length, a condition

observed in the outgroup species *Ctenosciaena gracilicirrhus* and *Pachyurus schomburgkii*, and in the ingroup species *Plagioscion auratus*, *Plagioscion magdalenae* and *Plagioscion montei*. The apomorphic state, with 11% of the standard length or higher, was identified in *L. breviceps*, *M. ancylodon*, *Isopisthus parvipinnis*, *Cynoscion acoupa*, *C. jamaicensis*, *C. leiarchus* and in the ingroup species *Plagioscion squamosissimus*, and *P. ternetzi*. In the analysis, the plesiomorphic state appears as a reversal to the clade comprised of *P. auratus*, *P. magdalenae*, and *P. montei*.

3.1.2. Pectoral Fin

[7] Pectoral-fin length: (0) short, falling short of vertical through the anus; (1) long, reaching vertical through the anus [ci = 0.50; ri = 0.50; Steps = 2].

Larimus breviceps, *Plagioscion magdalenae* and *P. montei*

In specimens of the Sciaenidae, the pectoral girdle always includes two extrascapulars, the posttemporal, supracleithrum, cleithrum, scapula, four radials, corachoid, and two postcleithra (Sasaki, 1989). It is in contact dorsally with the neurocranium through the posttemporal. Dermal bones are joined by the cleithrum, supracleithrum, postcleithra 1 and 2, and the lepidotrichia (Topp and Cole, 1968). Pectoral-fin length was a character used by Sasaki (1989), who considered the fin to be long when it reaches or extends beyond the anal-fin origin. This character was modified by Casatti (2000), who considered the fin to be long when its tip reaches the anus, and observed this apomorphic state in *P. magdalenae* and *P. montei* (*Plagioscion* sp. n. b sensu Casatti, 2000).

The present study follows the character description by Casatti (2000), and considers the pectoral fin to be long when the distal tip of the largest ray reaches the anus. Pectoral-fin length is a diagnostic character very frequently used to identify *Plagioscion* species. Few Sciaenidae species have a long pectoral fin that reaches or extends beyond the anus. Species with spindle-shaped and elongated bodies, such as specimens of the genus *Cynoscion*, have pectoral fin relatively short compared to the body with nearly always the same length as the pelvic fin (Keith et al., 2000). *Lonchurus* has a very long pectoral fin with upper radius filamentous reaching the caudal peduncle (Chao, 2002). Among the analyzed species in the present study, only *Larimus breviceps*, *P. magdalenae*, and *P. montei* have long pectoral fins. Thus, short pectoral fin was considered to be plesiomorphic. *Plagioscion squamosissimus*, *P. auratus*, and *P. ternetzi* have the plesiomorphic shape for this character, as well as the outgroup species, except for *L. breviceps*. Long pectoral fin represents an apomorphic condition shared by *P. magdalenae* and *P. montei*, and is a homoplasy for *L. breviceps*.

3.1.3. Pelvic Fin

[8] Pelvic-fin length: (0) short, falling short of anus; (1) long, reaching the anus [ci = 0.33; ri = 0; Steps = 3].

Larimus breviceps, *Plagioscion auratus*, and *P. magdalenae*

In the Sciaenidae, the pelvic girdle is formed by the thin and triangular basipterygium, ornamented with keels that support lepidotrichia, corresponding to the rays. Pelvic rays generally comprise a hard and indivisible ray and five branched rays (Sasaki, 1989). For this character, the distal tip of the largest ray either reaching anus or falling short of anus was considered. The relative pelvic-fin length is of great value for the taxonomy of *Plagioscion* (Campos, 1942; Ferreira et al., 1998; Keith et al., 2000; Casatti, 2005) where it is typically related to standard length. In ingroup analysis, long pelvic fin reaching anus was observed in the species *P. auratus* and *P. magdalenae*, thus justifying the polarity of this character as apomorphic. The plesiomorphic state, represented by short pelvic fin, was recorded in all other outgroup species, except in *L. breviceps*, and in species *P. squamosissimus*, *P. montei*, and *P. ternetzi*. In the final topology, pelvic long fin represents a synapomorphy for the clade *P. auratus*, *P. magdalenae*, with a reversal in *P. montei*. The derived condition is also shared independently with *L. breviceps*.

3.1.4. Anal Fin

[9] Length of the second anal-fin spine: (0) long, more than 80% of the length of the longest anal-fin ray; (1) short, up to 70% of the length of the longest anal-fin ray [ci = 0.50; ri = 0.80; Steps = 2].

Cynoscion acoupa, *C. jamaicensis*, *C. leiarchus*, *Isopisthus parvipinnis*, *M. ancyloodon*, *Plagioscion squamosissimus*, and *P. ternetzi*

In Sciaenidae, the anal fin is formed by pterygiophores and lepidotrichia. Generally, the first pterygiophore, larger and more robust, supports the two first anal-fin spines, which is typical of Sciaenidae, since few species have an anal-fin spine (Sasaki, 1989). Thus the presence of two anal-fin spines is a synapomorphy for Sciaenidae (Sasaki, 1989). In percoids the presence of three anal-fin spines is considered to be plesiomorphic and reduced spines are probably not homologous in several families (Johnson, 1984). The length of second anal-fin spine is used to identify *Plagioscion* species (Campos, 1942; Ferreira et al., 1998; Keith et al., 2000; Casatti, 2005) albeit the variation in the length of this spine among populations (Casatti, 2005). Casatti (2000) distinguished two conditions for the second anal-fin spine, considering second anal-fin spine strong and long to be plesiomorphic, and short and weak to be apomorphic.

For the present study, we chose to treat two characters related to the second anal-fin spine: length and thickness (character 10). Second anal-fin spine length was measured from the base of the spine to its distal tip, and is considered long when it goes beyond 80% of the length of the longest soft fin ray (Fig. 4A). In taxa analyzed in this study, the second anal-fin spine length in the ingroup species *Plagioscion ternetzi* and *P. squamosissimus*, despite variability, was not more than 70% of the length of the longest soft anal-fin ray, and was usually smaller than this limit (Fig. 4B); this condition is shared by the outgroup species (*I. parvipinnis*, *M. ancyloodon*, *C. acoupa*, *C. jamaicensis*, and *C. leiarchus*) and ingroup species *Plagioscion ternetzi* and *P. squamosissimus*. Hence the derived condition is present in a larger group, with a reversal in the clade comprised of *P. auratus*, *P. magdalenae*, and *P. montei*.

[10] Second anal-fin spine thickness: (0) robust, four times the thickness of the first soft anal-fin ray; (1) narrow, up to twice the thickness of the first soft anal-fin ray [ci = 0.50; ri = 0.80; Steps = 2].

Cynoscion acoupa, *C. jamaicensis*, *C. leiarchus*, *Isopisthus parvipinnis*, *Macrodon ancyloodon*, and *Plagioscion squamosissimus*

In addition to the trends of reductions of the anal-fin length and number of rays (character 9), the decreased spine thickness was also observed, as discussed by Casatti (2000), although slightly modified in the present paper. Generally, when the spine is long, it is also more robust; however, some species might present the spine robust and short, such as *Umbrina canosai* and *P. ternetzi*. Second spine thickness was treated as a different character from length in this study. The robust spine (Fig. 4A) regarded as plesiomorphic, here standardized to four times the thickness of the first soft anal-fin ray, was observed in the outgroup species *Ctenosciaena gracilicirrhus* and *Larimus breviceps*, and in the ingroup species *P. auratus*, *P. magdalenae*, *montei*, and *P. ternetzi*. The apomorphic condition, proposed as up to twice the thickness of the first soft anal-fin ray (Fig. 4B), is a synapomorphy for the outgroup species *I. parvipinnis*, *M. ancyloodon*, *Cynoscion acoupa*, *C. jamaicensis*, and *C. leiarchus*. In the ingroup, the apomorphic state is a homoplasy restricted to *P. squamosissimus*, and represents a character of taxonomic importance owing to its easy viewing and to its role in species diagnosis, given that the second ray length might be variable, albeit always narrow.

[11] Relation of anal-fin pterygiophore with haemal spine of the caudal vertebra: (0) distal tip of the first anal-fin pterygiophore not connected to the haemal spine of the second caudal vertebra; (1) distal tip of the first anal-fin pterygiophore linked to the tip of the haemal spine of the second caudal vertebra [ci = 0.33; ri = 0.33; Steps = 2].

Macrodon ancyloodon, *Pachyurus schomburgkii*, *Plagioscion squamosissimus* and *Plagioscion ternetzi*

The outgroup species *Ctenosciaena gracilicirrhus* and *Larimus breviceps* presented the first anal-fin pterygiophore as long and in contact

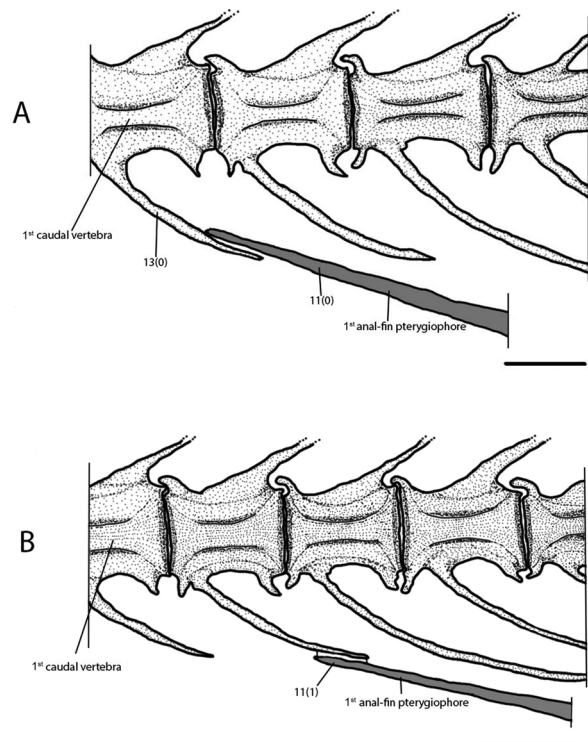


Fig. 5. Left lateral view of the caudal vertebrae and first pterygiophore of the anal fin of: A) *Plagioscion magdalenae*, MPEG 2795, 108.84 mm SL; B) *P. squamosissimus* MPEG 2947, 107.87 mm SL. Bar = 2 mm. Characters 11 and 13, in parentheses the respective states.

with the haemal arch of first and second caudal vertebrae. This characteristic was also described for the genus *Scienops* (Topp and Cole, 1968), and is considered to be a method of identifying the first caudal vertebra. Other outgroup species such as *Isopisthus parvipinnis*, *Macrodon ancyloodon*, *C. acoupa*, *C. leiarchus*, and *C. jamaicensis*, although they do not present the first anal-fin pterygiophore as long as in *Ctenosciaena gracilicirrhus* and *Larimus breviceps*, also have this element relatively close to the haemal arch of the first caudal vertebra (Fig. 5A). Regarding this condition as plesiomorphic, the species *Plagioscion squamosissimus*, *P. ternetzi*, *Macrodon ancyloodon*, and *Pachyurus schomburgkii* were observed to have an apomorphic condition; as for these species, the first anal-fin pterygiophore was reduced and with its distal tip joined by ligament tissue to the haemal spine of the second caudal vertebra (Fig. 5B). Considering the reduction in the first pterygiophore to be apomorphic, it can be inferred that this character is a synapomorphy between *Plagioscion squamosissimus* and *Plagioscion ternetzi*, and as homoplasy in *Pachyurus schomburgkii*.

3.1.5. Vertebrae

[12] Number of caudal vertebrae: (0) 15; (1) 14; (2) 13 [ci = 1.0; ri = 1.0; Steps = 2].

State 1; *Isopisthus parvipinnis*, *Plagioscion auratus*, *P. magdalenae*, *P. montei*, *P. squamosissimus*, and *P. ternetzi*. State 2; *Cynoscion acoupa*, *C. jamaicensis*, *C. leiarchus*, and *Macrodon ancyloodon*

Sciaenidae specimens generally have 10 thoracic and 15 caudal vertebrae, and with few exceptions (Chao, 2002) this pattern is regarded as the primitive state for percoids (Johnson, 1984; Sasaki, 1989). Species rarely vary from the total of 25 vertebra (Chao, 2002), but it is possible to observe differences in the proportion between thoracic and caudal vertebra. This feature is used as a diagnosis at family (Skogsberg, 1939), genus, and species levels (Sasaki, 2001), mainly in the identification of young individuals and in ontogenetic studies (Richards, 2005; Fahay, 2007). Caudal vertebrae are counted from the vertebra exhibiting pre- and post-zygapophysis, neural,

haemal, and based on the complete formation of haemal arch and haemal spine (Topp and Cole, 1968; Sasaki, 1989).

In the present paper, all specimens presented a total number of 25 vertebrae, and there was variation in the proportion of caudal and thoracic vertebrae. Three states were identified in relation to the number of caudal vertebrae. The plesiomorphic state is represented by the presence of 15 caudal vertebrae, observed in specimens of *Ctenosciaena gracilicirrhus* and *Pachyurus schomburgkii*. Apomorphic state 1 is identified by the presence of 14 caudal vertebrae, found in all specimens of *Plagioscion* and in the outgroup species *Isopisthus parvipinnis*. State 2 is represented by 13 caudal vertebrae, a condition shared by the species *Macrodon ancylodon*, *Cynoscion acoupa*, *C. jamaicensis*, and *C. leiarchus*. This character was treated as additive, since the gradual reduction of vertebrae represents a series of transformations, with the lowest number representing the most derived state. The condition of 14 caudal vertebrae is a synapomorphy for a large group involving part of the outgroup species (*Larimus breviceps*, *Isopisthus parvipinnis*, *Macrodon ancylodon*, *Cynoscion acoupa*, *C. jamaicensis*, and *C. leiarchus*) plus all *Plagioscion* species. The outgroup species *L. breviceps* was coded as unknown (-) in the matrix, owing to the identification of 15 caudal vertebrae in this analysis instead of 14 vertebra indicated in previous analyses (Fahay, 2007; Itagaki et al., 2007; Ninin, 2008). Therefore, as the vertebra count is a consistent diagnostic character in the Sciaenidae, the species *L. breviceps* possibly presents intraspecific variation for this character, making it impossible to discriminate the states, although the parsimony method suggests the occurrence of 14 vertebrae.

[13] Sagittal process posterior in the haemal arch of the first caudal vertebra forming a joint for the first anal-fin pterygiophore: (0) absence; (1) presence [ci = 0.50; ri = 0; Steps = 2].

Larimus breviceps and *Plagioscion auratus*

Caudal vertebrae are identified by the presence of pre- and postzygapophysis, neural and haemal, and owing to the complete formation of haemal canal and haemal spine (Topp and Cole, 1968; Sasaki, 1989). Haemal arch and haemal spines of caudal vertebrae are formed by the convergence of parapophysis. As described in character 10, in the plesiomorphic state, the first anal-fin pterygiophore is long and, in some species, in contact with the haemal arch of the first caudal vertebra (Fig. 5A). However, the haemal arch is a derived state in the species *L. breviceps* and *P. auratus*, represented by a sagittal process long and posterior which helps form the joint of the first anal-fin pterygiophore (Fig. 6). Considering the distance between both species in the final topology, the most parsimonious interpretation is that these are

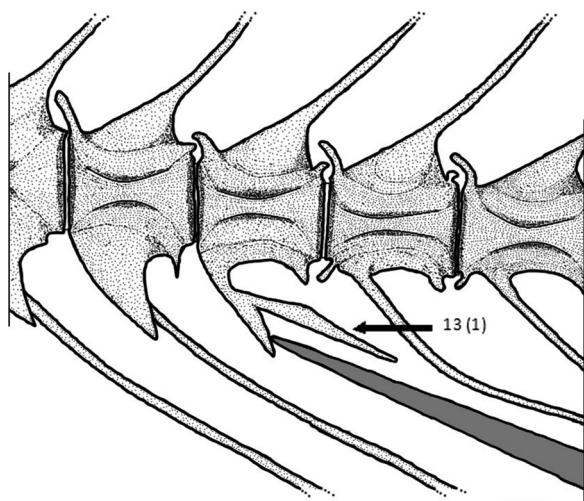


Fig. 6. Left lateral view of vertebrae of *Plagioscion auratus*, MPEG 9868, 157.83 mm SL. Bar = 2 mm. Highlighted, posterior sagittal process in the haemal arch of the first caudal vertebra; character 13, in parentheses the derived state.

independent events. Their occurrence in *P. auratus* represents an important diagnostic character. This character was regarded by Casatti (2000) as the most derived state with the first caudal vertebra having pleural ribs. In this study, no modifications related to pleural ribs described by Casatti (2000) were observed, and this character was analyzed separately. Due to the distance between species that share the derived state, the presence of this character in both species, *L. breviceps* and *P. auratus*, represents a homoplasy.

[14] Number of posterior vertebrae on the last anal-fin pterygiophore: (0) up to nine vertebrae; (1) ten vertebrae [ci = 1.0; ri = 1.0; Steps = 1].

Plagioscion magdalenae and *P. montei*

In the present study, all specimens examined had a count of 25 vertebrae; however, there were differences in the position of fins in relation to vertebrae, especially considering the anal fin, which influences the number of vertebra counted from the end of fin pterygiophores. In specimens of genus *Plagioscion* a difference in number of vertebrae posterior to the last anal-fin pterygiophore was observed. *Plagioscion montei* and *P. magdalenae* had ten caudal vertebrae and *P. squamosissimus*, *P. auratus*, and *P. ternetzi* had nine. As a polarity determination for this character, despite the variable number of vertebrae, all outgroup specimens had nine or less vertebrae, thus limiting the plesiomorphic state to no more than nine caudal vertebrae. Therefore, ten caudal vertebrae were considered to be a synapomorphy for *P. montei* and *P. magdalenae*.

[15] Number of vertebrae after the last dorsal-fin pterygiophore: (0) six or more; (1) five [ci = 0.5; ri = 0.66; Steps = 2].

Pachyurus schomburgkii, *Plagioscion auratus*, *P. magdalenae* and *P. montei*

In Sciaenidae, the dorsal fin is typically long with a deep notch between the anterior portion (with spines) and the posterior portion, with soft rays. Only *Isopisthus* has both portions well separated (Sasaki, 1989, 2001; Chao, 2002). The posterior part of dorsal fin, also called second dorsal, is much longer than the anal fin. The dorsal fin has a similar constitution as the anal fin, with pterygiophores supporting spines and soft rays. In *Plagioscion* specimens, a slight difference in the position of the end of the dorsal fin was observed in relation to the anal-fin base. In order to avoid using morphometric data, we opted for counting caudal vertebrae posterior to the last dorsal-fin pterygiophore, where a variation between five and six vertebrae was observed. In the outgroup analysis, despite observing a variation of five to seven vertebrae posterior to the last dorsal-fin pterygiophore, only *Pachyurus schomburgkii* possessed a count of five vertebrae, and a decreasing pattern in the number of caudal vertebrae was observed, which reflects a posterior-most positioning of the end of the dorsal fin even though it is not representatively sized. Therefore, the plesiomorphic state was identified by a vertebra count ranging from six to seven vertebrae after the last dorsal-fin pterygiophore, condition which is present in *Ctenosciaena gracilicirrhus*, *Macrodon ancylodon*, *Isopisthus parvipinnis*, *Cynoscion acoupa*, *Cynoscion jamaicensis*, *Cynoscion leiarchus*, and in the ingroup species *Plagioscion squamosissimus* and *P. ternetzi*. The apomorphic state was limited to five or less caudal vertebrae, joining the ingroup *Plagioscion auratus*, *P. magdalenae* and *P. montei*, being in homoplasy with *Pachyurus schomburgkii*.

3.1.6. Caudal Skeleton

[16] Relationship between first and second hypurals: (0) separated; (1) fused [ci = 1.0; ri = 0; Steps = 1].

Plagioscion auratus

The sciaenid caudal skeleton generally consists of five hypurals, one parhypural with a distinct parhypurapophysis, two uroneurals, three epurals, one urostyle, and preural centra 1 and 2 located in front of the urostyle (Gosline, 1961; Topp and Cole, 1968; Sasaki, 1989). The hypural is a bone compressed as a joining plate with the posterior part of the urostyle. Caudal-fin rays are connected to hypural bones.

The caudal skeleton structure of the specimens analyzed follows the

proposal by Gosline (1961), with primitive aspect among percoids, with a maximum number of independent bones (Topp and Cole, 1968). Fused elements in the caudal skeleton are not common in the Sciaenidae. Topp and Cole (1968) mentioned that large specimens of *Sciaenops* might have hypurals 4 and 5 fused. Sasaki (1989) mentioned that despite a rich variety of caudal-fin shapes in Sciaenidae, the formation of the hypurals is remarkably even. Although he found hypurals 1 and 2 fused in specimens of *Nebris microps*, he decided not to use this character. Casatti (2000) described hypurals 1 and 2 fused as an autapomorphy for the species *P. auratus*. In the present study, hypurals 1 and 2 fused were only found in *P. auratus*; therefore, the condition of fusion of hypurals 1 and 2 was considered to be apomorphic and hypurals widely separated was considered to be plesiomorphic. Hence the apomorphic state represented an autapomorphy of the species, as observed in the aforementioned paper.

3.1.7. Otolith

[17] Shape of ostium of *sulcus acusticus*: (0) tadpole-shaped; (1) oval; (2) spherical [ci = 0.66; ri = 0.75; Steps = 3].

State 1: *Cynoscion acoupa*, *C. jamaicensis*, *Isopisthus parvipinnis*, *Macrodon ancylodon*, *Plagioscion auratus*, *P. magdalenae*, *P. montei*; State 2: *P. squamosissimus* and *P. ternetzi*

Otoliths are found in the auditory bulla, located in the ventral part of the neurocranium, on both sides of the head (Chao, 2002). They are formed by calcified concretions and are located in the inner ear of the teleost fishes, occurring in three symmetric pairs, *Lapillus*, *Asteriscus*, and *Sagitta* (Corrêa and Vianna, 1992). These structures play a role in the functions of balance and hearing in fishes (Bastos, 1990). There are consistent hypotheses that associate the presence of relatively large otoliths in species which produce sounds, and the examples cited are Sciaenid specimens (Monteiro et al., 2005; Pansard, 2009), which possess large otoliths apparently to detect sounds from other individuals of the species (Assis, 2003). Due to its size, the otolith is frequently used in ecological, paleontological, and taxonomic studies (Worthman, 1979; Begg et al., 2005). It is an important character that varies widely among fish species, helping to distinguish genera and species (Adams, 1940; Torno, 1976; Worthman, 1979; Gaemers, 1984). For Sasaki (2001) the correct identification of some genera and species is not possible without examining otoliths.

In the medial inner side of the otolith is the *sulcus acusticus*, a “grooved” imprint that is generally described as “tadpole-shaped” in Sciaenidae (Chao 2002), formed by *ostium* (posterior portion), *sulcus* (intermediate part, which separates *ostium* from tail), and tail (posterior part of the *sulcus acusticus*) (Bastos, 1990; Corrêa and Vianna, 1992). The shape of this *sulcus* generally helps in a correct specific identification (Chao 2002). Sasaki (1989) did not work with *ostium* shapes but with the location of its posterior portion and the shape of the sulcus tail.

For the present study, otolith *ostium* and tail shapes were analyzed, as described in the cited literature (Hecht, 1987; Bastos, 1990; Corrêa and Vianna, 1992). In the specimens analyzed, the geometric shape of otoliths varied from ellipsoid to oval, with a *sulcus acusticus* of the heterosulcoid type, with excision narrow and anterodorsal. Regarding *ostium* shape, three states were identified: tadpole-shaped, spherical, and oval. As a polarity determination of *ostium* shapes, the tadpole-shape was considered to be plesiomorphic (Fig. 7A) since it was observed in the outgroup species *Ctenosciaena gracilicurrus*, *Pachyurus schomburgkii*, *Larimus breviceps*, and as a reversal in *Cynoscion leiarchus*. The following shapes were considered to be derived conditions: oval, identified as state 1 (Fig. 7B), and spherical, identified as state 2 (Fig. 7C). In the literature consulted (Hecht, 1987; Bastos, 1990; Corrêa and Vianna, 1992), other *ostium* shapes are described, but with no signs that might indicate a possible series of transformations, hampering the establishment of any type of ordering for the states of this character. Therefore, we chose to treat this character as non-additional. State 1 was identified in outgroup species *Macrodon ancylodon*, *Isopisthus parvipinnis*, *Cynoscion jamaicensis*, and *C. acoupa*. Chao (2003) mentioned

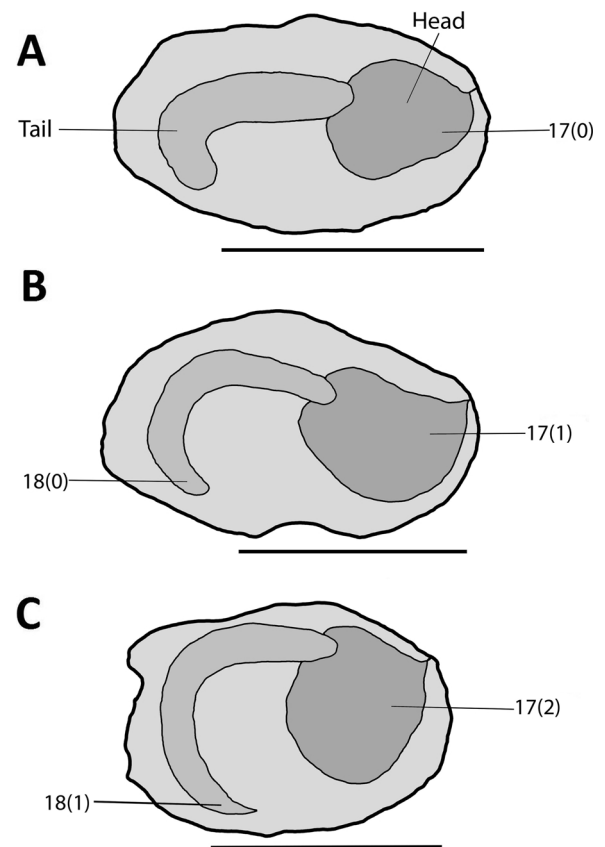


Fig. 7. Internal medial surface of *Sagitta* otolith of: A) *Cynoscion leiarchus*, MZUSP 68857, 101.98 mm SL; B) *Plagioscion montei*, MPEG 808, 146.7 mm SL; C) *Plagioscion ternetzi*, MZUSP 21145, 162.68 mm SL. Bar = 5 mm. Highlighted in character 17 and 18, in parentheses the respective states.

that *Cynoscion* species generally possesses an oval-shaped *ostium*. State 1 arises as a synapomorphy for a large group that involves outgroup species (*Cynoscion acoupa*, *C. jamaicensis*, *C. leiarchus*, *Isopisthus parvipinnis*, *Macrodon ancylodon*) and all *Plagioscion* species. State 2 represents a more inclusive synapomorphy of the genus *Plagioscion* in the clade comprised of *P. squamosissimus* and *P. ternetzi*.

[18] Distal end of *sulcus acusticus* tail: (0) circular; (1) narrowly elongate [ci = 0.50; ri = 0.50; Steps = 2].

Plagioscion auratus, *P. squamosissimus* and *P. ternetzi*

In the *Sagitta* otolith of Sciaenidae specimens, the *sulcus* tail is generally well curved. Variations in this curvature were commented by Sasaki (1989) as being of taxonomic and phylogenetic importance. For the present study, significant changes in the *sulcus acusticus* curvature were observed only in the outgroup; however, a notable modification in the distal end of the *sulcus acusticus* was identified in the ingroup. *Sagitta* otoliths of the species *P. squamosissimus*, *P. auratus*, and *P. ternetzi* had the distal end of the *sulcus* tail narrowly elongate, as opposed to that found in other species, in which this structure is circular. Sasaki (1989) does not mention variations in the *sulcus*, which can be quite narrowly elongate, but indicates that the *sulcus* tail expanded to an oval shape as an apomorphic character for some genera such as *Macrodon*, *Otolithoides*, *Chrysochir*, and *Otolithes*. This apomorphy was also observed in the present study in specimens of *Macrodon ancylodon*; however, as this is an isolated manifestation in the outgroup, we chose to code it in the matrix as non-comparable (-). In the analysis of the distal end of the *sulcus* tail in *Plagioscion*, the condition of rhombus shaped, not exactly circular, was considered to be plesiomorphic (Fig. 7B); this condition was observed in *P. magdalenae*, and *P. montei* in the outgroup and ingroup. Notably narrowly elongate tail was regarded as the apomorphic state (Fig. 7C), as observed in *P. squamosissimus*, *P. auratus*,

and *P. ternetzi*, representing a derived condition for these species. The parsimony analysis showed that narrowly elongate tail, according to the topology obtained in this paper, represents a synapomorphy for the genus *Plagioscion* with reversals in *P. magdalenae* and *P. montei*.

[19] Granulated ridge on the outer surface of the otolith: (0) absence; (1) presence [ci = 1.0; ri = 0; Steps = 1].

Plagioscion auratus

In the *Sagitta* otolith, the configurations identified in the *sulcus acusticus* provide the main information used in ecological and systematic analyses. Some papers (Fisher et al., 1981; Chao, 2002) also describe the outer surface of the otolith, which is generally convex, varying between smooth, undulated, or with granulations. The granulated state is described in some genera such as *Atractoscion*, *Pseudotolithus*, *Argyromus*, and *Sciaena*, whose outer otolith surface is granulated and with irregular ridges (Fisher et al., 1981). Among the specimens analyzed in the present paper, only *P. auratus* specimens did not have outer surface smooth or slightly undulated; instead, it had the formation of a granulated ridge in the posterior portion. Hence, the absence of a granulated ridge was regarded as a plesiomorphic state as opposed to its presence. The condition found in *P. auratus* is an autapomorphy which has not been described previously for this species, and it represents a character of taxonomic relevance, as it might aid in diagnosis and in the correct identification of the species.

3.1.8. Swimbladder

[20] Horn-shaped appendages of the swimbladder: (0) absent; (1) present; [ci = 1.0; ri = 0; Steps = 1].

Cynoscion acoupa, *C. jamaicensis*, *C. leiarchus*, *Isopisthus parvipinnis*, *Larimus breviceps*, *Macrodon ancylodon*, *Pachyurus schomburgkii*, *Plagioscion auratus*, *Plagioscion magdalenae*, *Plagioscion montei*, *Plagioscion squamosissimus*, and *Plagioscion ternetzi*

The swimbladder in Sciaenidae specimens is well developed and typically occupies the entire length of the abdominal cavity, but does not extend posteriorly beyond the first anal pterygiophores. Swimbladder morphology shows informative changes in Sciaenidae due to different combinations of vibratory musculature responsible for emitting sounds (Trewavas, 1962; Chao, 1978; Sasaki, 1989). Swimbladder vibration is considered to be a sound production mechanism owing to the action of specialized muscles, related to reproductive behavior (Locascio and Mann, 2005).

Swimbladder morphology is quite diversified in Sciaenidae, ranging from simple shape, with no appendages or inner chambers, to shapes with large branched appendages and one or more inner chambers interconnected. They are further distinguished by their branching pattern, number, and length. Sasaki (1989) considered a simple, elongate and carrot-shaped swimbladder with no inner divisions or appendages to be plesiomorphic for Sciaenidae (Fig. 8A). Ontogenetic information is consistent with the hypothesis that a simple swimbladder lacking appendages is the plesiomorphic condition for the Sciaenidae (Yamada, 1973).

The presence of horn-shaped appendages in the anterior region of the swimbladder (Fig. 8B) was described by Chao (1978), who observed this pattern in specimens of *Cynoscion*, *Macrodon*, *Isopisthus*, and *Plagioscion*. These appendages might vary in length and shape depending on genus and species. Thus, this character might be used for taxonomic identification. Sasaki (1989) mentioned that the presence of a pair of horn-like appendages, arising at the anterior end of the swimbladder (cephalic region) is apparently a very common modification in Sciaenidae, and proposes this character as a synapomorphy for the subfamily Cynoscioninae.

In the present paper, the character referring to swimbladder appendages is considered to be apomorphic, and was observed in *Larimus breviceps*, *Pachyurus schomburgkii*, *Isopisthus parvipinnis*, *Macrodon ancylodon*, *Cynoscion acoupa*, *C. leiarchus*, *C. jamaicensis*, *C. striatus*, *Plagioscion squamosissimus*, *Plagioscion auratus*, *Plagioscion magdalenae*, *Plagioscion montei*, and *Plagioscion ternetzi*. Swimbladder appendages

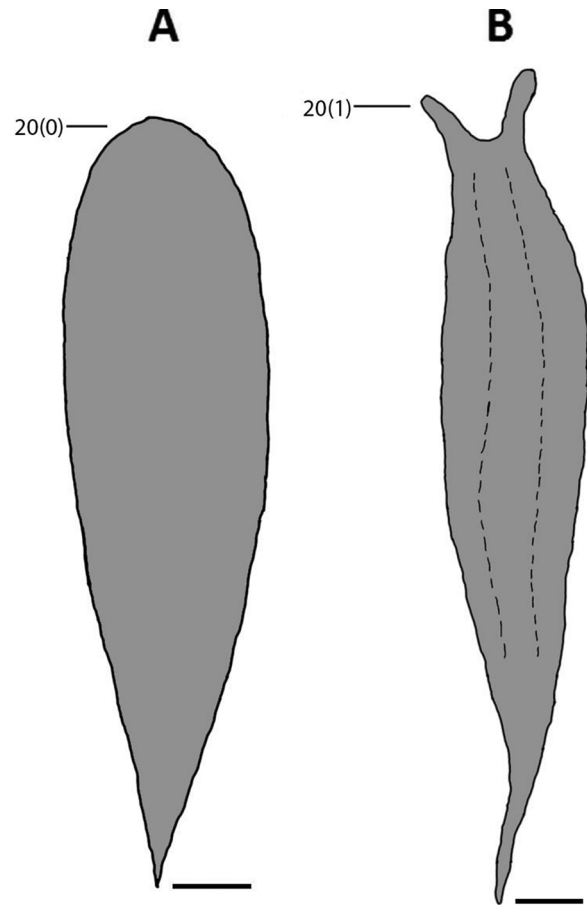


Fig. 8. Dorsal view of the swimbladder of: A) *Ctenosciaena gracilicirrhus*, MZUSP 68699, 88.82 mm SL; B) *Macrodon ancylodon*, MCT 5537, 142.54 mm SL. Bar = 5 mm. Highlighted the character 20, between parentheses the respective states.

vary in length and shape depending on the species; they can be straight, curved, hook-like, and looping back to the cephalic region of the swimbladder. *Larimus breviceps* was the species with the smallest appendages, and considered by Sasaki (1989) as belonging to a distinct subfamily (Lariminae). A simple swimbladder was observed in *Ctenosciaena gracilicirrhus*, and this shape was considered plesiomorphic, and this species had the highest phylogenetic distance.

[21] Length of the anterior appendages of the swimbladder: (0) short, up to 10 % of the swimbladder length, (1) long more than 15% of the swimbladder length [ci = 0.50; ri = 0.50; Steps = 2].

Cynoscion acoupa, *C. jamaicensis*, *C. leiarchus*, *Isopisthus parvipinnis*, *Macrodon ancylodon*, *Plagioscion auratus*, *P. magdalenae*, *P. montei*, *P. squamosissimus*

This character was described based on observations of a discrepancy in length of appendages described in character 20. Although horn-like appendages prolonged in the swimbladder cephalic region might vary in shape and size among genera and species. *Pachyurus schomburgkii*, *L. breviceps*, and *Plagioscion ternetzi* were observed to have the lowest values for appendage length.

In an attempt to determine the polarity of this character, appendages with length up to 10% of the swimbladder length, from the cephalic tip to the posterior end, excluding further appendages, are regarded as plesiomorphic (Fig. 9A). Considering that this condition is present in the outgroup species *L. breviceps*, the appearance of swimbladder appendages in Sciaenidae might have arisen from the simple swimbladder shape, followed by short appendages, which derived to long appendages, which in turn, derived to more complex and branched shapes, common in several taxa of the family.

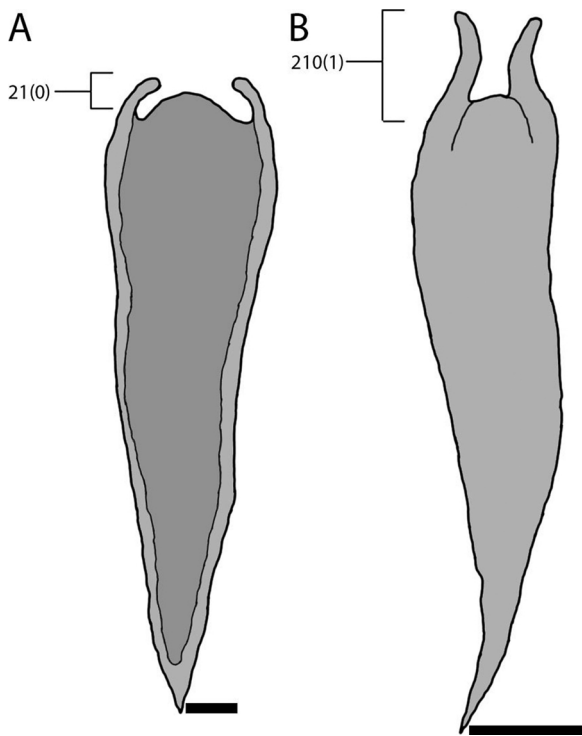


Fig. 9. Dorsal view of the swimbladder of: A) *Plagioscion ternetzi*, MZUSP 21145, 222.04 mm SL; B) *Cynoscion jamaicensis*, MCT 3536, 107.58 mm SL. Bar = 5 mm; Highlighted the character 21, between parentheses the respective states.

The apomorphic state, here represented by longer appendages of more than 15% of the swimbladder length (Fig. 9B) is a widely spread condition in the taxa analyzed (*Cynoscion acoupa*, *C. jamaicensis*, *C. leiarchus*, *Isopisthus parvipinnis*, *Macrodon ancylodon*, *Plagioscion auratus*, *P. magdalenae*, *P. montei*, *P. squamosissimus*), despite the variety of shapes. In the ingroup, the plesiomorphic state is present only in *P. ternetzi* as a reversal and it is a character of taxonomic relevance, as it contributes to the diagnosis of *P. ternetzi* compared to *P. squamosissimus*, which are highly similar in their external morphology, and which, according to the cladogram obtained, comprise a monophyletic group.

[22] Shape of swimbladder appendages: (0) straight extended anteriorly; (1) looped back to the cephalic portion of the swimbladder [ci = 1.0; ri = 1.0; Steps = 1].

Plagioscion auratus, *P. magdalenae*, *P. montei*, *P. squamosissimus* and *P. ternetzi*

This character is related to the previously described ones (20 and 21). Horn-like appendages observed in the taxa studied showed variations in shape. Carrot-shaped appendages, anteriorly expanded, a condition which is here considered to be plesiomorphic (Fig. 10A), are shared by the species *I. parvipinnis*, *M. ancylodon*, *C. acoupa*, *C. jamaicensis*, *C. striatus*, and *Pachyurus schomburgkii*, all of which belong to the outgroup. The genus *Pachyurus* has other swimbladder shapes, including a simple swimbladder (Casatti, 2000). *Cynoscion leiarchus* possesses crescent-shaped appendages. *Larimus breviceps* has appendages extremely short, but carrot-shaped. Studies that have addressed swimbladder morphology in *Plagioscion* refer to horn-like swimbladder appendages, but these appendages differ from other shapes found in the *Cynoscioninae*. The swimbladder appendages found in *Plagioscion* are described here as looping back to the swimbladder cephalic region. The state of appendages looping back to the cephalic region and with their distal end spiraling, is an apomorphy (Fig. 10B) shared only by *Plagioscion* species, representing a new synapomorphy for the genus.

[23] Swimbladder appendages forming lateral tube-like structures from the swimbladder anterior end to the posterior end: (0) absent; (1)

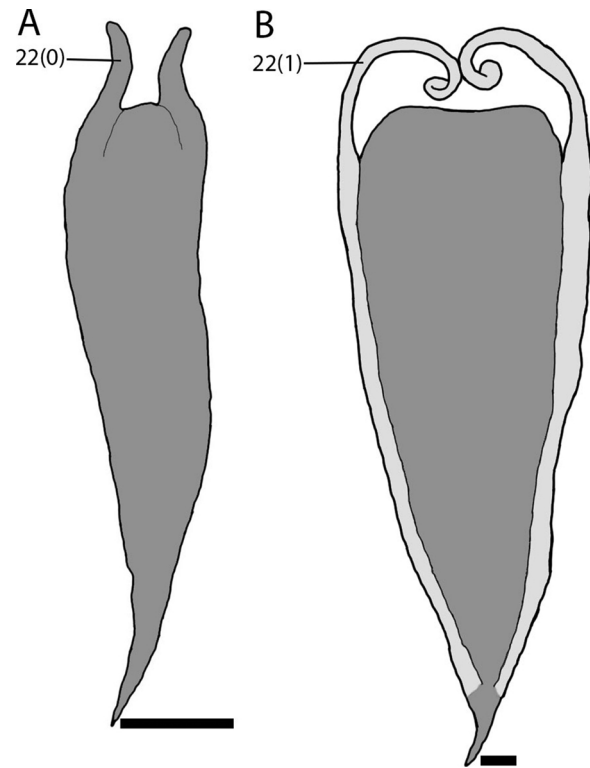


Fig. 10. Dorsal view of the swimbladder of: A) *Cynoscion jamaicensis*, MCT 3536, 107.58 mm SL; B) *Plagioscion auratus* MPEG 9866, 266.42 mm SL. Bar = 5 mm; Highlighted the character 22, between parentheses the respective states.

present; [ci = 0.50; ri = 0.80; Steps = 2].

Cynoscion leiarchus, *Plagioscion auratus*, *P. magdalenae*, *P. montei*, *P. squamosissimus*, and *P. ternetzi*

This character is related to the previously described characters 20, 21, and 22. In addition to swimbladder appendages, these structures form lateral tubules throughout the extension of the swimbladder in *Plagioscion* species. This condition was also observed in *Cynoscion leiarchus*. Sasaki (1989) described the appendages present in *P. auratus* as tube-like along the swimbladder rims, arising on its posterior portion, and considered the same character to be present in *Otholithoides* and *Micropogonias*. However, he suggested that they do not represent homologous conditions. The same author pointed out that this condition is not present in *P. ternetzi*, and described this taxon as having only a dorsal and ventral ridge along the swimbladder sides, not forming tube-like structures. In the present study, the swimbladder of *P. ternetzi* was also observed to have appendages forming lateral tube-like structures (confirmed by the internal morphological analysis of the swimbladder), a condition shared among all *Plagioscion* species analyzed, representing a synapomorphy for this genus, shared as a homoplasy with *C. leiarchus*. Consequently, the swimbladder of *P. ternetzi* only differs from its congeners by having shorter appendage length, discussed in character 21.

The polarity of this character, based on the outgroup, allowed for regarding the presence of a pair of appendages on the anterior portion of the swimbladder as plesiomorphic compared to the swimbladder with appendages forming tube-like structures (Fig. 11C and D), although it is an apomorphic condition compared to the simple swimbladder with no tubules (Fig. 11A and B).

The analysis of the internal morphology of the swimbladder in the outgroup species, with appendages, demonstrated a trend in forming lateral tubules (*I. parvipinnis*, *M. ancylodon*, *Cynoscion acoupa*, *C. jamaicensis* and *C. leiarchus*). *Cynoscion jamaicensis* specimens had the internal swimbladder wall smooth, but with thicker tissue on the sides.

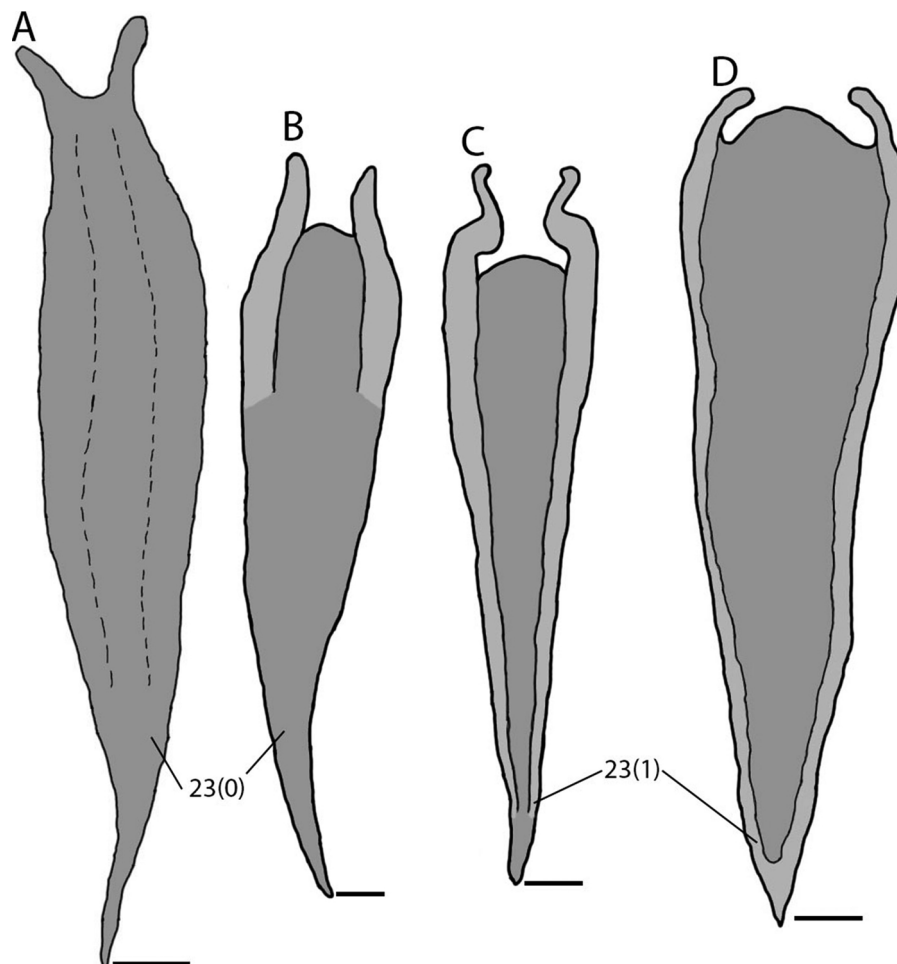


Fig. 11. Dorsal view of the swimbladder of: A) *Macrodon ancylodon* MCT 5537, 142.54 mm SL (tracejado significa entalhe); B) *Cynoscion acoupa* MZUSP 68719, 134.91 SL; C) *Cynoscion leiarchus* MZUSP 68857, 112.64 mm SL; D) *Plagioscion ternetzi*, MZUSP 21145, 222.04 mm SL. Bar = 5 mm. Highlighted the character 23, between parentheses the respective states.

Specimens of *M. ancylodon* had an inner ridge on the swimbladder sides (Fig. 11A), at the dorsal and ventral portions, but did not form tubules. There seems to be a beginning of inner tubule formation in the swimbladder appendages of *C. acoupa* and *I. parvipinnis*, on the swimbladder sides slightly before its half-length (Fig. 11B). Specimens of *C. leiarchus* had lateral tubules totally formed (Fig. 11C), similar to *Plagioscion* species, being different in the shape of the anterior extensions (character 20). Sasaki (1989) mentioned that the trend in forming tube-like structures on the swimbladder seems to be present in *Cynoscion*, as the same condition is found in *C. nebulosus*. Darovec (1983) described *C. nothus* as having a carrot-shaped swimbladder internally, *C. regalis* as having a ridge described as parasagittal partitions on the sides, and proposed *C. nebulosus* as more derived based on characters of external and osteological morphology.

3.1.9. External Morphology

[24]. Morphology of lateral line scales: (0) simple scales; (1) modified scales [ci = 1.0; ri = 1.0; Steps = 2].

Plagioscion auratus, *P. magdalenae*, *P. montei*, *P. squamosissimus*, and *P. ternetzi*

The lateral line, easily visible and continuous to the opercular, extending to the end of the caudal fin, was regarded as a synapomorphy for the Sciaenidae (Sasaki, 2001). According to Trewavas (1977), this feature would be a potential specialization related to sound reception. However, this character derived independently in other perciform families (Sasaki, 1989). The continuous lateral line is not an exclusive characteristic of Sciaenidae (Darovec, 1983; Chao, 1995). Greenwood

(1976) mentioned this form of lateral line as being also present in Centropomidae. However, in Sciaenidae specimens, the lateral line scales generally have the same size as the adjacent body scales (Fig. 12A), although the pore on the scales is quite pronounced and easily viewed. The presence of modified lateral line scales, formed by large perforated scales completely covered by small scales, is a character used as diagnosis for the genus *Plagioscion* (Fig. 12B and C), described previously by Chao (1978) and proposed as synapomorphy for the genus by Sasaki (1989; based on the analysis of *P. auratus* and *P. ternetzi*), is considered to be homoplasy in *Panna microdon* and *Otholithoides pama*, and is not present in other *Panna* or *Otholithoides* species.

In the present paper, no specimens of the genera *Panna* and *Otholithoides* were analyzed; however, in the taxa examined, the apomorphic state of modified scales was observed in all species of the *Plagioscion* genera and is not present in any outgroup species, thus reinforcing the hypothesis that this character is considered to be a synapomorphy for the *Plagioscion* genus and that the occurrence in *Panna microdon* and *Otholithoides pama* is an independent event.

[25] Scaling on the second dorsal fin: (0) no scales or scales on the base; (1) densely scaled [ci = 1.0; ri = 0; Steps = 1].

Plagioscion ternetzi

Sciaenidae specimens generally have a long dorsal fin, deeply notched, which separates the first portion with hard spines from the second portion with soft rays (separated in *Isopisthus*). The first part has 6–13 spines and the second part has one spine and generally 20–35 soft rays (Nelson, 2006). The dorsal-fin portion with soft rays is also known as the second dorsal and is generally without scales or slightly scaled only

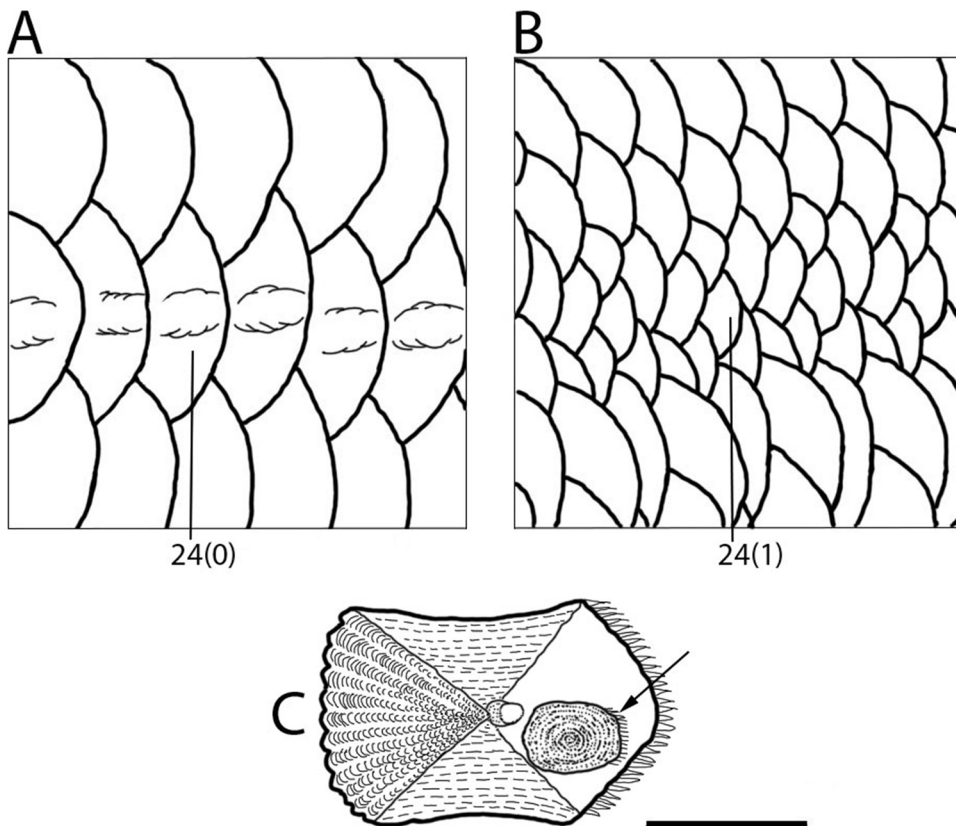


Fig. 12. Scales of the lateral line of: A) *Larimus breviceps*, MZUSP 69139, 108.51 mm SL; B) *Plagioscion ternetzi* MZUSP 21145, 230.14 mm SL. C) Lateral line scale, arrow indicates micro-scale that overlaps perforated scale, *P. ternetzi* MZUSP 21145, 222.04 mm SL. Bar = 2 mm. Highlighted the character 24, between parentheses the respective states.

on its base, which provides a good view for counting rays.

In this study, the fin is considered densely scaled when several rows of cycloid scales are present, forming a thick wedge which makes it difficult to view the base of rays on the second dorsal. This condition was only observed in *P. ternetzi*. This character represents an autapomorphy of taxonomic relevance for *P. ternetzi*, as it contributes to the quick identification of this species. Casatti (2005), who reviewed the genus *Plagioscion*, highlighted that it might be difficult to distinguish *P. ternetzi* from *P. squamosissimus* in young individuals using morphometry and meristic data. Due to similarities, pre-eminent scaling on the second dorsal fin would be the primary character that separates both species, which form a monophyletic group.

[26] Distance from anus to anal fin: (0) Up to 28% of the length of the head, (1) More than 33% of the length of the head [ci = 0.50; ri = 0.66; Steps = 2].

Larimus breviceps, *Plagioscion auratus*, *P. magdalenae*, and *P. montei*

The standardized distance from anus to the anal-fin origin, considering its percentage compared to the length of the head, is a character frequently used in the diagnosis of *Plagioscion* species (Campos, 1942; Ferreira et al., 1998; Keith et al., 2000; Casatti, 2005). In the outgroup analyzed, there was a short distance between the anus and the anal-fin origin, and this character was identified as plesiomorphic. The derived state, represented by an anus farther from the anal-fin origin (represented here by a distance longer than 33% of the length of the head), was observed in *Larimus breviceps*, *Plagioscion auratus*, *P. magdalenae*, and *P. montei*, representing a synapomorphy for these three *Plagioscion* species and a homoplasy in relation to *Larimus breviceps*.

[27] Length of anal-fin base: (0) long, more than 90% of the depth of caudal peduncle; (1) short, up to 68% of the depth of caudal peduncle [ci = 0.50; ri = 0; Steps = 2].

Plagioscion montei and *P. squamosissimus*

This character was obtained using the measurement of the anal-fin base, measured between the first and last anal spines, treated as

percentage in relation to the depth of caudal peduncle. *Plagioscion* have few characters without data overlap; however, specimens of *P. squamosissimus* and *P. montei* were observed to have length of anal fin relatively smaller than their congeners. As a means to limit values without overlaps, the measure of the anal-fin base was compared to the depth of the caudal peduncle.

The plesiomorphic state observed in the outgroup species *Ctenosciena gracilicirrus*, *Larimus breviceps*, *Pachyurus schomburgkii*, *Cynoscion acoupa*, and *Cynoscion jamaicensis* indicates a caudal-fin length very similar to the depth of the caudal peduncle. Only the species *Macrodon ancylodon*, *Isopisthus parvipinnis*, and *Cynoscion leiarchus* had the anal-fin length relatively longer than caudal peduncle; however, it is typical of these species to have a relatively large anal fin.

The ingroup species *P. auratus*, *P. magdalenae*, and *P. ternetzi* also had length of caudal fin quite similar to that observed in the outgroup, and the plesiomorphic state was outlined as having the length of the anal fin greater than 90% of the depth of the caudal peduncle. The apomorphic state was defined as short when measuring up to 68% of the depth of caudal peduncle, generally smaller than this limit. The derived state represents a shared character between the species *P. squamosissimus* and *P. montei*; however, the parsimony analysis shows that they represent distinct origins.

[28] Restriction to freshwater: (0) life in marine or brackish waters; (1) restricted to freshwater. [ci = 0.5; ri = 0.8; steps = 2].

Pachyurus schomburgkii, *Plagioscion auratus*, *Plagioscion magdalenae*, *Plagioscion montei*, *Plagioscion squamosissimus*, and *Plagioscion ternetzi*

Sciaenidae is a group of fishes which primarily occur in salt waters (plesiomorphic state), with some groups (*Aplodinotus*, *Pachypops*, *Pachyurus*, *Plagioscion*, *Boesemania* and *Petipinnis*; Casatti, 2002b) secondarily restricted to freshwater (apomorphic state). Phylogenetic relationships between the genera of this family are still uncertain; however, apparently, the invasion and subsequent restriction to freshwater environment occurred in different ways and at different moments among the groups (Casatti, 2002b; Boeger and Krisky, 2003; Lovejoy

et al., 2006). In the present paper, restriction to life in freshwater arises as a synapomorphy for *Plagioscion* species, with an independent occurrence in *Pachyurus schomburgkii*, according to the hypotheses already mentioned.

3.2. Description and Discussion of Data

3.2.1. Clade 1: Genus *Plagioscion* (Fig. 13)

The genus *Plagioscion* is a monophyletic group with the following hypothesized phylogenetic relationship: ((*P. squamosissimus* + *P. ternetzi*) (*P. magdalenae* (*P. montei* + *P. magdalenae*))). This clade had a decay index of two steps and is supported by five synapomorphies. Of those, four are exclusive to this group: intercalar with ventral projection, *sulcus acusticus* tail with end narrowly elongated, swimbladder appendages looping back to swimbladder cephalic region and lateral-line scales modified. As an ambiguous synapomorphic character, this clade has swimbladder appendages forming tube-like lateral structures. Characters 4 (intercalar projecting downward) and 24 (lateral-line scales modified) were originally proposed by Sasaki (1989) as synapomorphies for the genus *Plagioscion*; however, they were homoplastic. Nevertheless, in the present study, due to a smaller outgroup than that used by Sasaki (1989), these characters were only found in *Plagioscion*, supporting the previous hypothesis that these characters are regarded as a synapomorphy for the genus. Casatti (2000) proposed five synapomorphies for the genus, also mentioned in the taxonomic review of the genus (Casatti, 2005): parasphenoid with dorsal projection, fewer than five ossified epipleural ribs, the presence of horned swimbladder appendages, the presence of a dark axillary spot and tolerance to freshwater. For the present paper, dorsal projection of the parasphenoid was not considered to be a synapomorphy for the genus; according to the final topology obtained, this character arose as a synapomorphy for a larger group (*Larimus breviceps*, *Isopisthus parvipinnis*, *Cynoscion* spp., *Macrodon ancylodon*, and *Plagioscion*), as a reversal of the clade comprised of *Isopisthus parvipinnis*, *Cynoscion* spp., *Macrodon ancylodon*. The presence of a pair of swimbladder appendages was found widely in the outgroup; thus, it did not represent a synapomorphy for the genus in this analysis. Following Sasaki (1989) in outgroup selection, the character of reduced epipleural ribs was not included in the analysis since it is a synapomorphy for the tribe Cynoscionini (Sasaki, 1989). The presence of a dark axillary spot was not included in this paper, as this character was observed to be widely present in the outgroup, including the rooted taxa. Hence, it is not regarded as apomorphic in this analysis. With a larger outgroup, it would certainly be possible to include this character in the analysis; however, it would not be informative for a better resolution in the ingroup. Restriction to freshwater was regarded as apomorphic, and it appears as a synapomorphy for *Plagioscion* with independent occurrence in *Pachyurus schomburgkii*.

3.2.1.1. Synapomorphies.

- 1 Intercalar (4): (0 > 1) projecting downward, forming part of auditory bulla. Exclusive state.
- 2 Distal end of *sulcus acusticus* tail (18): (0 > 1) narrowly elongate. Exclusive state.
- 3 Shape of swimbladder appendages (22): (0 > 1) looping back to the cephalic portion of swimbladder. Exclusive state.
- 4 Swimbladder appendages forming lateral structures tube-like from the anterior end to the posterior end of swimbladder (23): (0 > 1) present. Homoplasy with *Cynoscion leiarchus*.
- 5 Morphology of lateral-line scales (24): (0 > 1) modified scales. Exclusive state.

3.2.2. Clade 2: *Plagioscion squamosissimus* and *P. ternetzi*

The clade is supported by three synapomorphies; two of them are exclusive: parasphenoid dorsal projection dagger-shaped and distal end of first anal-fin pterygiophore connected to the end of haemal spine of

the second caudal vertebra. As an exclusive synapomorphy, it has *sulcus acusticus* orbicular. The relationship between the species forming a monophyletic group has never been reported in the literature, only a morphometric and meristic similarity. The clade presented a decay index of 2 steps. Although *P. squamosissimus* derives from the Rio Amazonas basin and *P. ternetzi* derives from the Ríos Paraná-Paraguay-Uruguay system, the characters investigated support the hypothesized consistent relationship.

3.2.2.1. Synapomorphies.

- 1 Shape of the parasphenoid dorsal projection (3): (0 > 1) projection dagger-shaped. Homoplasy with *P. montei*.
- 2 Relation of anal-fin pterygiophore with haemal spine of the caudal vertebra (11): (0 > 1) distal end of first anal-fin pterygiophore connected to haemal spine of second caudal vertebra. Homoplasy with *Macrodon ancylodon* and *Pachyurus schomburgkii*.
- 3 Shape of *ostium* of *sulcus acusticus* (17): (1 > 2) orbicular. Exclusive state.

3.2.2.2. *Autapomorphy* of *Plagioscion ternetzi*. 1. Scaling on the second dorsal fin (25): (0 > 1) densely scaled. Exclusive character.

3.2.2.3. *Reversal* of *Plagioscion ternetzi*. 1. Length of appendage horn-like (21): (1 > 0) short, up to 10% of the swimbladder length.

3.2.3. Clade 3: *Plagioscion auratus*, *P. montei*, and *P. magdalenae*

This clade has a decay index of 2 steps, has resolution (*P. auratus* (*P. montei* + *P. magdalenae*)), and is supported by three non-exclusive synapomorphies: pelvic-fin long reaching anus, six or more vertebra after the last dorsal-fin pterygiophore, and distance from anus to anal fin more than 33% of the length of the head. The clade presented two reversals: the distance between posterior end of supraoccipital and insertion of the first dorsal-fin spine with up to 8% of standard length, and length of second anal-fin spine more than 80% of the length of largest anal-fin ray. This clade was previously described by Casatti (2000), supported by a reverse character described as the spine of the second anal-fin ray strong and long. As described in character 9, we chose to treat spine thickness and length as distinct characters. External and internal morphology characters, analyzed in the present study, reflect the phylogenetic proximity of the species. All the species in this clade have distribution in the Rio Amazonas basin.

3.2.3.1. Synapomorphies.

- 1 Pelvic-fin length (8): (0 > 1) long, reaching anus. Homoplasy with *Larimus breviceps*.
- 2 Number of vertebrae after the last dorsal-fin pterygiophore (15): (0 > 1) five. Homoplasy with *Pachyurus schomburgkii*.
- 3 Distance from the anus to anal fin (26): (0 > 1) more than 33% of the length of the head. Homoplasy with *Larimus breviceps*.

3.2.3.2. Reversals.

- 1 Distance from the posterior tip of the supraoccipital to the insertion of the first spine onto the dorsal fin (6): (1 > 0) Up to 8% PL.
- 2 Length of the second anal-fin spine (9): (1 > 0) Long, more than 80% of the length of the largest anal-fin ray.

3.2.3.3. *Autapomorphies* of *Plagioscion auratus*.

- 1 Fusion of hypurals 1 and 2 (16): (0 > 1) fused. Exclusive state.
- 2 Ridge granulated on the external otolith surface (19): (0 > 1) presence. Exclusive state.

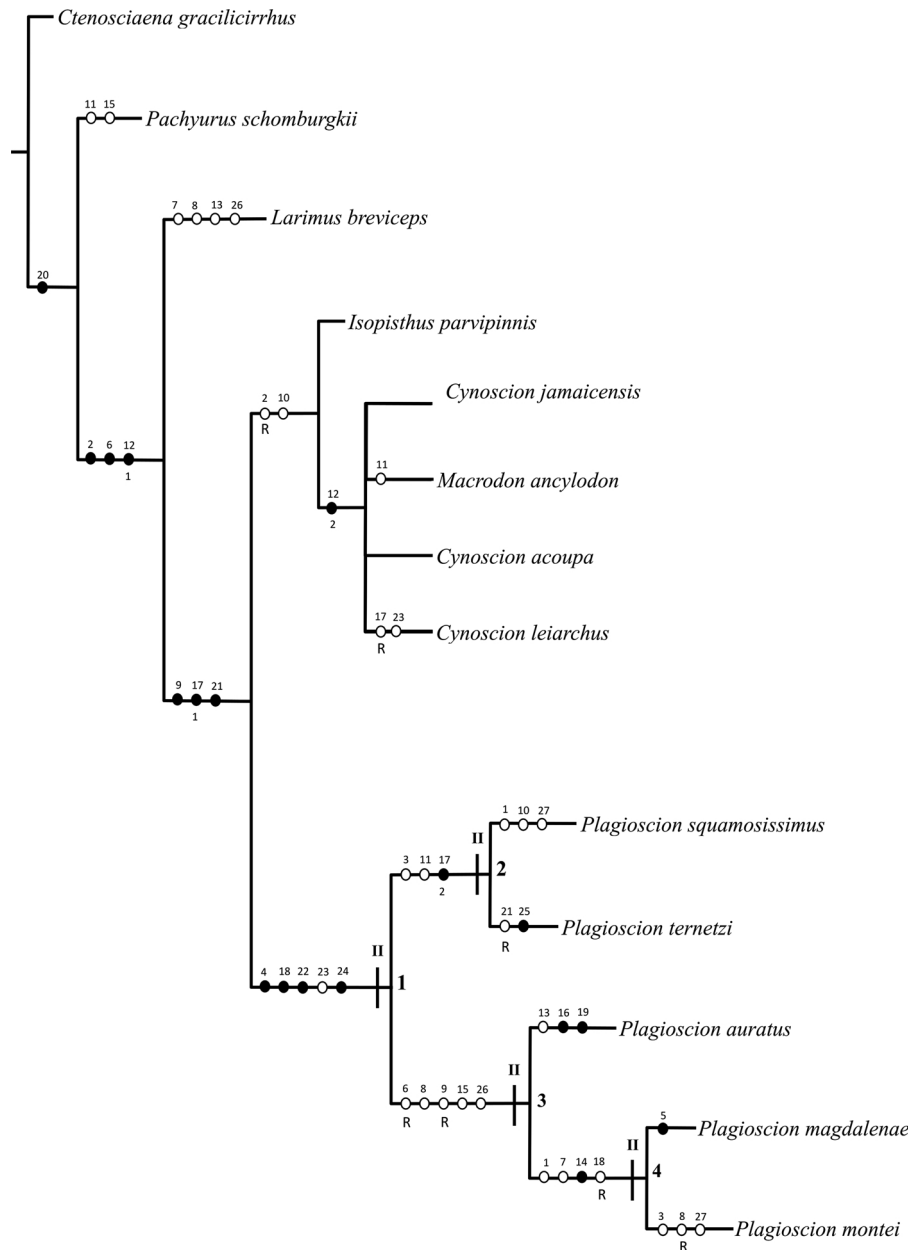


Fig. 13. More parsimonious cladogram (steps = 50, CI = 0.60 and IR = 0.72) representing the phylogenetic relationships of *Plagioscion* species. Arabic numerals in major source represent the clade number; Roman numerals represent the decay rate. Synapomorphies represented by circles: white circles = homoplasias; black circles = unique synapomorphies. R = Reversions.

3.2.4. Clade 4: *Plagioscion montei* and *Plagioscion magdalenae*

This group is supported by three synapomorphies. Of those, one is exclusive to this group:

10 posterior vertebrae on the last anal-fin pterygiophore. As non-exclusive synapomorphic characters, the clade has: basisphenoid not connected to dorsal portion of parasphenoid and pectoral fin long, reaching vertical through anus. As a reversal, the clade had distal end of *sulcus* tail circular. The species *P. magdalenae* and *P. montei* were grouped as sister groups by Casatti (2000) as one clade supported by the two synapomorphies mentioned above: basisphenoid separated from parasphenoid and pectoral long. The clade presents a decay index of 2 steps, the same value indicated for other clades in the ingroup. This monophyletic group corroborates Casatti (2000); additionally, new characters are added in the present study.

3.2.4.1. Synapomorphies.

- 1 Relationship between parasphenoid and basisphenoid (1): (0 > 1) (1) separated in posteroventral rim. Homoplasy with *P. squamosissimus*
- 2 Pectoral-fin length (7): (0 > 1) long and reaching vertical through anus. Exclusive character.
- 3 Number of posterior vertebrae on the last anal-fin pterygiophore (14): (0 > 1) 10 vertebra. Exclusive character.

3.2.4.2. Reversals. 1. Distal end of *sulcus* tail (18): (1 > 0) circular.

3.2.4.3. Autapomorphies of *Plagioscion magdalenae*. 1. Interorbital width (5): (0 > 1) narrow, up to 17 % of length of the head.

3.2.4.4. Reversal of *P. montei*. 1. Pelvic-fin length (8): (1 > 0) (0) short, falling short of anus.

4. Comments

The first appreciation of *Plagioscion* in a phylogenetic paradigm was performed by Sasaki (1989), who proposed a relationship for Sciaenidae genera. *Plagioscion* was proposed as a natural group, represented by *Plagioscion* sp., *P. auratus*, and *P. ternetzi*, sister group of the clade (*Cynoscion Macrodon Isopisthus*). These taxa and five other genera (*Atractoscion*, *Macropsinosa*, *Kathala*, *Panna*, and *Boesemania*) comprised clade “D4”, supported by the synapomorphy “a pair of appendages horn-like arising at anterior end of swimbladder,” confirmed and discussed in the present paper (see characters 20–22). Cooke et al. (2011), based on molecular data, hypothesized a relationship between *Plagioscion* species, regarding this genus as monophyletic and also including two cryptic species, resulting in (*Plagioscion auratus* (*P. magdalenae* (*P. ternetzi* (*Plagioscion* sp. 1 (*P. squamosissimus*^(Meta River) *P. squamosissimus*))) (*Plagioscion* sp. 2 *P. montei*))). Recently, Lo et al. (2015) re-submitted a Scienidae phylogeny based on molecular data, where *Plagioscion* (*P. auratur* (*P. surinamenses* (*P. ternetzi* *P. squamosissimus*))) is a sister group of (*Pachyurus bonariensis Pachypops fourcroy*) and both comprise “Lineage 6”. As seen, Lo et al. (2015) did not include *Plagioscion magdalenae* and *Plagioscion montei*; on the other hand, they considered *Plagioscion surinamensis* to be valid without providing a justification, as this species was considered by Casatti (2005) to be a synonym to *P. squamosissimus*. Both proposals, by Cooke et al. (2011) and Lo et al. (2015), are different from each other, and different from the present paper (Fig. 13); however, they converge in the relationships between *P. squamosissimus* and *P. ternetzi* as sister species (present paper and Lo et al. 2015) and partially in Cooke et al. (2011) who presents a clade (*Plagioscion* sp. 1 (*P. squamosissimus*^(Meta River) *P. squamosissimus*)) as a sister group to *P. ternetzi*. In the present analysis, specimens from the studies by Cooke et al. (2011) and Lo et al. (2015) were not analyzed. However, the relative divergence between the three topologies is possibly due to the difference in methodologies used. In the present analysis, all *Plagioscion* clades presented a decay index of 2, which is relatively robust, considering that any change in its topology requires a relative increase in the number of steps, resulting in a less parsimonious tree.

5. Conclusions

The *Plagioscion* (Sciaenidae; Eupercaria) genus is a natural group of Neotropical freshwater fishes, composed by five species, and supported by five synapomorphies. Its internal relationships can be represented by the topology ((*P. squamosissimus* + *P. ternetzi*) (*P. magdalenae* (*P. montei* + *P. magdalenae*))).

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