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Phylogenomics of the Neotropical fish family Serrasalmidae with a novel intrafamilial classification (Teleostei: Characiformes)

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ABSTRACT

The Neotropical fish family Serrasalmidae comprises 16 extant genera and 101 species widespread through major Neotropical rivers with relevant importance for regional fisheries and aquaculture. The monophyly of Serrasalmidae and the recognition of three main clades are recurrent between morphological and molecular phylogenies. However, both intergeneric and interspecific relationships within each of those clades remain uncertain. Here, we used 81 terminals of 69 species (68%) and all 16 genera of Serrasalmidae to sequence 1553 loci of ultraconserved elements (UCEs), multiple nuclear loci widely applied in phylogenetic studies, and performed maximum likelihood, Bayesian, and species tree analyses. We obtained highly supported phylogenies in all applied methods corroborating the monophyly of Serrasalmidae and the three-clade hypotheses herein proposed as two subfamilies and two tribes: (Colossomatinae (Serrasalminae (Myleini + Serrasalmini))). Morphological features for each subfamily involve the absence (Colossomatinae) or presence (Serrasalminae) of a pre-dorsal spine. Morphological diagnoses among tribes include the pre-dorsal spine being continuous (Myleini) or discontinuous (Serrasalmini) relative to the first unbranched dorsal-fin ray. Our results highlight the complexity of the relationships especially the non-monophyly of *Myleus*, *Mylesinus*, *Myloplus*, *Tometes*, and *Utiaritichthys* within Myleini, as well as of *Serrasalmus* and *Pristobrycon* within Serrasalmini.

1. Introduction

Serrasalmidae is a family of Neotropical freshwater fishes comprising 17 genera, including †*Megapiranha* from the Upper Miocene, and 101 valid species (Fricke et al., 2020a), represented by the popularly known “pacus”, “piranhas” and the large “tambaquis”. Serrasalmids are broadly distributed through all major South American river basins (Jégu, 2003; Nico et al., 2018) and possess a wide diversity of dietary specializations such as carnivory, herbivory and lepidophagy, resulting in extremely polymorphic morphological teeth shape and arrangement, which have being largely used as diagnostic characters in taxonomy and systematics of the family (Goulding, 1980; Nico et al., 2018; Nico and Taphorn, 1998; Sazima, 1983; Sazima and Machado, 1990).

Although the majority of nominal species of Serrasalmidae were described in the 19th century (Jégu et al., 2004), the first classification was proposed by Eigenmann (1915), who recognized two subfamilies within Characidae: Serrasalminae, composed by species with one row of teeth on premaxilla and Myleinae (Mylinae – *sic*), composed by species with two rows of teeth in the premaxilla, including *Catoprion*. Posteriorly, Géry (1972) allocated *Catoprion* in Catoprioninae. However, these classifications did not represent phylogenetic relationship among genera, e.g. *Metynnis* and *Catoprion* were subsequently obtained as closely related to species with a single row of premaxillary teeth (Cione et al., 2009; Machado-Allison, 1983; Ortí et al., 2008; Thompson et al. 2014).

By the end of the 20th century, the taxonomic status of serrasalmids was discordant being ranked either as a family (Géry, 1972, 1976; Géry et al., 1987; Jégu and dos Santos, 1988) or a subfamily of Characidae (Buckup, 1998; Jégu, 2003, 2004; Merckx et al., 2000). In the last decades, morphological studies suggested the placement of serrasalmids as closer to Alestidae and Characidae (Mirande, 2010, 2009), while

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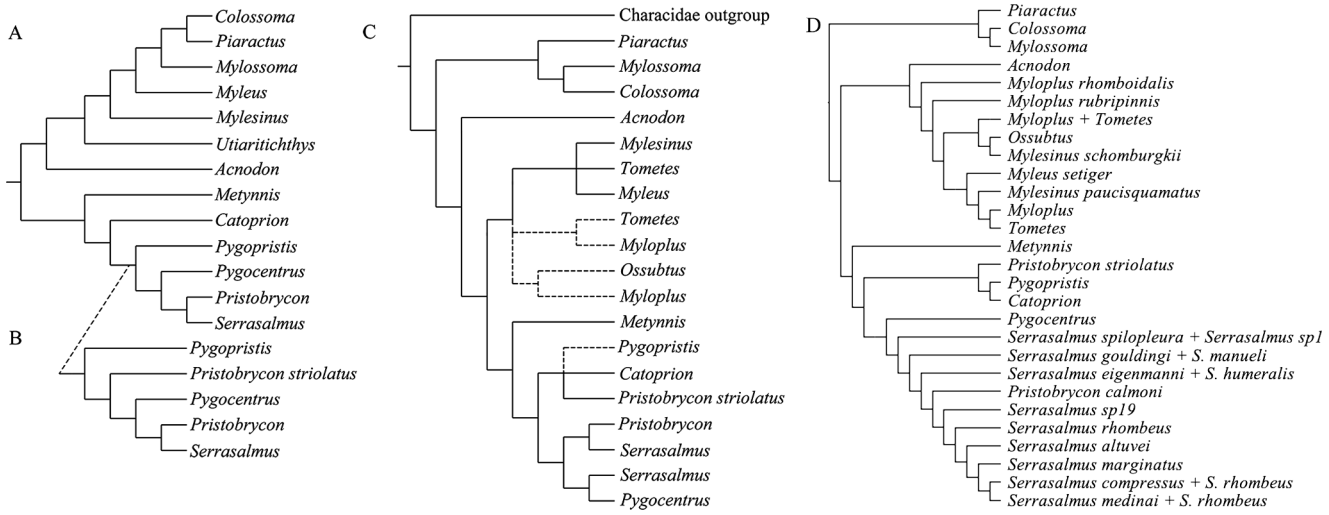

Fig. 1. Previous intergeneric hypotheses of Serrasalmidae, modified from original publications. (A) Morphological phylogeny (Machado-Allison, 1982); (B) Morphological phylogeny (Machado-Allison et al., 1989). (C) Molecular phylogenies based on mtDNA (Ortí et al., 2008, 1996); dotted lines indicate differences among studies. (D) Molecular phylogeny based on 10 nuclear and mitochondrial loci (Thompson et al. 2014).

multilocus and total-evidence studies agree with the placement of Serrasalmidae closer to the superfamily Anostomoidea and relatives (Burns and Sidlauskas, 2019; Calcagnotto et al., 2005; Mirande, 2019; Oliveira et al., 2011). Recent phylogenomic studies, however, obtained the family as closely related to Hemiodontidae and Cynodontidae (Arcila et al., 2017) or sister to Hemiodontidae (Betancur et al., 2018). The first cladistic analysis of the family was performed by Machado-Allison (1982, 1983) based on an extensive study of morphological characters, and the monophyly of Serrasalmidae (as subfamily Serrasalminae) was supported by 27 synapomorphies. The family contained two major clades referred as A and B. The "lineage A" was composed by most of Myleinae proposed by Eigenmann (1915) (*i.e. Acnodon*, *Colossoma*, *Mylesinus*, *Myleus*, *Mylossoma*, *Piaractus*, and *Utiaritichthys*), and the "lineage B" was constituted by all "piranhas" *sensu* Eigenmann (1915) (*i.e.*, *Pristobrycon*, *Pygocentrus*, *Pygopristis* and *Serrasalmus*) with addition of *Metynnis* and *Catoprion* (Fig. 1A). However, further investigations revealed paraphyletic genera, such as *Myleus* in the "lineage A" (Machado-Allison, 1983) and *Pristobrycon* in the "lineage B" (Machado-Allison et al., 1989) (Fig. 1B).

The first molecular analysis based on sequences of *12S* and *16S* mitochondrial ribosomal genes, included 37 species and all genera except *Ossubtus*, *Pygopristis*, and *Utiaritichthys* (Ortí et al., 1996). The species were divided in three rather than two major clades, with the first clade composed by *Colossoma*, *Mylossoma*, and *Piaractus*. In the second clade, the "*Myleus* clade", *Acnodon* was sister of two other groups that included *Myleus*, *Mylesinus* and a new genus (*Tometes sensu* Jégu et al., 2002), and the "true piranhas" group, composed by the remaining serrasalmids with both *Serrasalmus* and *Pristobrycon* as non-monophyletic (Fig. 1C). However, relatively low levels of sequence divergence among rRNA genes resulted in a poor interspecific resolution. These results were posteriorly corroborated by Ortí et al. (2008), analyzing 44 sequences of the mtDNA control region (*D-loop*) along with *12S* and *16S* sequences and 74 taxa, with *Utiaritichthys* absent. The three main clades were "pacu clade" as sister to all other serrasalmids, and the "*Myleus* clade" sister to the "piranha clade" (Fig. 1C). Consistent with previous results, the monophyly of *Myleus*, *Tometes*, *Pristobrycon*, and *Serrasalmus* were not supported (Ortí et al., 2008).

More recently, Thompson et al. (2014) performed a calibrated phylogenetic analysis using multilocus in Serrasalmidae including 38 nominal species (40% of the family diversity), also not including *Utiaritichthys*. The authors corroborated the monophyly of the three main clades and the non-monophyly of several taxa (e.g. *Pristobrycon*, *Tometes*, *Myloplus*, and *Serrasalmus rhombeus*) (Fig. 1D). They also suggested a more recent diversification of the "piranha clade" during the Miocene (20–15 Ma) compared to the other two clades that began to diversify during the Cretaceous (65–75 Ma). However, the "piranha clade" was much older than that proposed by Hubert et al. (2007) and Cione et al. (2009). The most recent phylogenomic approach of Characiformes used 12 serrasalmids and returned a distinct topology with *Piaractus* sister to all serrasalmids, and then three main clades: *Mylossoma/Colossoma*, the "*Myleus* clade" with *Acnodon*, *Myloplus*, *Myleus*, and *Ossubtus*, and the "piranha clade" with *Pristobrycon, Pygocentrus*, and *Serrasalmus* (Betancur et al., 2018).

Despite those several morphological and molecular studies dedicated to elucidate the phylogenetic relationships within Serrasalmidae, they focused either on morphological characters (Machado-Allison, 1983; Machado-Allison et al., 1989), a few molecular markers (Ortí et al., 1996, 2008; Thompson et al., 2014), or used few specimens in broader phylogenomic approaches (Betancur et al., 2018), and none of them included all serrasalmid genera. Here, we use ultraconserved elements (UCEs), extremely conserved nuclear regions shared among distinct groups along the tree of life (Faircloth et al., 2012). Since UCEs are highly conserved, they are used as target to probes so that the polymorphic flanking regions can be used to reconstruct phylogenies. Therefore, UCEs have been used at many comparison levels among organisms, from population to large groups, for all sort of animals, as for example invertebrates (Faircloth et al., 2015; Zhang et al., 2018a,b), amphibians (Streicher et al., 2018), fishes (Faircloth et al., 2013; Alfaro et al., 2018; Roxo et al., 2019), non-avian reptiles (Crawford et al., 2012, 2015), birds (Barker, 2017; Bruxaux et al., 2018; Smith et al., 2014), and mammals (McCormack et al., 2012), proving to be highly resolute and allowing the construction of strongly supported phylogenies. Studies with fishes had shown high resolution in both ancient and recent clades, as for actinopterygians (Faircloth et al., 2013), Neotropical cichlids (Burress et al., 2018), acantomorphs (Alfaro et al., 2018), and siluriforms (Roxo et al., 2019; Ochoa et al. 2020). As such, the use of UCEs in complex groups, as Neotropical freshwater fishes, is very promising.

Although a consensus exists for the monophyly of Serrasalmidae as well as the presence of three main clades, the persistence of polytomies, non-monophyletic groups, and the uncertain taxonomic status of several genera and subgroups still remain. Therefore, the present study aims to elucidate the relationships within Serrasalmidae using all genera and a large number of species in a phylogenomic analysis of thousands of ultraconserved elements.

Table 1
Taxon, voucher and locality information of the analyzed specimens of Serrasalmidae and related taxa.

Taxon	Museum number	Voucher number	Locality, basin	Coordinates	Country
Acnodon normani Gosline 1951	LBP 19140	77187	Rio Paranã, Tocantins basin	12°37′31″S 47°52′59″W	Brazil
Catoprion mento (Cuvier 1819)	LBP 7556	35626	Rio Cuiabá, Paraguay basin	16°11′39″S 55°48′25″W	Brazil
Catoprion absconditus Mateussi, Melo & Oliveira 2020.	LBP 21615	63665	Igarapé Au-au, Amazon basin	02°56′19″N 61°03′06″W	Brazil
Colossoma macropomum (Cuvier 1816)	LBP 12837	54043	Rio Tapajós, Amazon basin	04°16′49″S 59°59′26″W	Brazil
Colossoma macropomum (Cuvier 1816)	LBP 12838	54052	Rio Tapajós, Amazon basin	04°16′49″S 59°59′26″W	Brazil
Metynnis altidorsalis Ahl, 1923	LBP 9997	43151	Rio Pelehojo, Orinoco basin	07°32′22″N 66°08′31″W	Venezuela
Metynnis cuiaba Pavanelli, Ota & Petry 2009	LBP 8580	43371	Rio Paraguai, Paraguay basin	15°04′33″S 57°11′04″W	Brazil
Metynnis fasciatus Ahl 1931	LBP 3978	22824	Lagoa Fazenda Taboca, Araguaia basin	20°05′07″S 50°58′59″W	Brazil
Metynnis guaporensis Eigenmann 1915	UFRO-ICT 5396	6018	Igarapé Jatuarana, Madeira basin	08°49′55″S 64°02′55″W	Brazil
Metynnis hypsauchen (Müller & Troschel 1844)	LBP 18390	43152	Rio Pelehojo, Orinoco basin	07°32′22″N 66°08′31″W	Venezuela
Metynnis lippincottianus (Cope 1870)	LBP 5429	27164	Rio Jari, Amazon basin	00°56′00″S 52°32′30″W	Brazil
Metynnis longipinnis Zarske & Géry 2008	LBP 15530	63920	Rio Takutu, Amazon basin	03°17′57″N 59°55′38″W	Brazil
Metynnis luna Cope 1878	LBP 18398	42589	Rio Guamá, Amazon basin	01°34′00″S 47°09′51″W	Brazil
Metynnis luna Cope 1878	LBP 9394	43863	Rio Guamá, Amazon basin	01°34′00″S 47°09′51″W	Brazil
Metynnis maculatus (Kner 1858)	UFRO-ICT 5395	3986	Rio Jaciparaná, Madeira basin	09°17′03″S 64°23′57″W	Brazil
Metynnis mola Eigenmann & Kennedy 1903	LBP 8447	42354	Rio Paraguai, Paraguay basin	16°03′13″S 57°48′31″W	Brazil
Metynnis polystictus Zarske & Géry 2008	LBP 8015	37703	Rio Arinos, Amazon basin	14°08′21″S 56°04′19″W	Brazil
Mylesinus paraschomburgkii Jégu, Santos & Ferreira 1989	LBP 20484	80923	Rio Jari, Amazon basin	00°32′01″S 52°35′05″W	Brazil
Mylesinus paucisquamatus Jégu & Santos 1988	LBP 12839	53447	Rio Tapajós, Amazon basin	04°33′09″S 56°17′59″W	Brazil
Myleus altipinnis (Valenciennes 1850)	LBP 24011	91323	Rio Pandeiros, São Francisco basin	15°12′40″S 44°49′54″W	Brazil
Myleus micans (Lütken 1875)	LBP 17403	69141	Rio Francisco Dumont, São Francisco basin	17°19′10″S 44°17′13″W	Brazil
Myleus setiger Müller & Troschel 1844	LBP 16523	61481	Rio Xingu, Amazon basin	03°15′24″S 52°05′47″W	Brazil
Myloplus arnoldi Ahl 1936	LBP 20266	79748	Rio Apiacás, Amazon basin	10°19′45″S 56°59′17″W	Brazil
Myloplus torquatus (Kner 1858)	LBP 13827	57247	Rio Tapajós, Amazon basin	04°33′09″S 56°17′59″W	Brazil
Myloplus asterias (Müller & Troschel 1844)	LBP 9072	42585	Rio Guamá, Amazon basin	01°34′17″S 47°10′10″W	Brazil
Myloplus levis (Eigenmann & McAtee 1907)	ZUFMS-PIS 5567	01	Rio Indaiá Grande, upper Paraná basin	19°43′32″S 51°55′48″W	Brazil
Myloplus planquettei (Jégu, Keith & Le Bail 2003)	ANSP 179808	230	Yukanopito falls, Essequibo basin	01°54′53″N 58°31′14″W	Guyana
Myloplus rhomboidalis (Cuvier 1818)	ANSP 185981	10924	Rio Iriri, Amazon basin	03°50′32″S 52°44′03″W	Brazil
Myloplus rubripinnis (Müller & Troschel 1844)	UFRO-ICT Uncatalogued	5782	Madeira basin	–	Brazil
Myloplus schomburgkii (Jardine 1841)	OS 18990	PE10-044	Rio Nanay, Amazon basin	03°45′10″S 73°17′00″W	Peru
Myloplus ternetzi (Norman 1929)	ANSP 188689	1626	Lawa river, Maroni basin	03°19′31″N 54°03′48″W	Suriname
Myloplus tiete (Eigenmann & Norris 1900)	INPA 53243	53243	Córrego Caracu, upper Paraná basin	22°40′00″S 53°15′00″W	Brazil
Mylossoma acanthogaster (Valenciennes 1850)	LBP 24311	91508	Rio Sardinata, Catatumbo basin	08°39′25″N 72°37′46″W	Colombia
Mylossoma albiscopum (Cope 1872)	LBP 12089	51722	Rio Madeira, Amazon basin	08°51′42″S 64°03′49″W	Brazil
Mylossoma aureum (Spix & Agassiz 1829)	UFRO-ICT 19346	10260	Igarapé Jatuarana, Madeira basin	08°45′54″S 64°02′39″W	Brazil
Mylossoma duriventre (Cuvier 1818)	LBP 3741	21919	Rio Negro, Paraguay basin	19°34′33″S 56°14′49″W	Brazil
Mylossoma unimaculatum (Steindachner 1908)	LBP 12745	41182	Rio Araguaia, Tocantins basin	13°18′37″S 50°36′47″W	Brazil

(*continued on next page*)

Table 1 (*continued*)

Taxon	Museum number	Voucher number	Locality, basin	Coordinates	Country
Mylossoma sp. (*sensu* Mateussi *et al.* in prep.)	LBP 18873	75056	Laguna del Castilleros, Orinoco basin	07°30′50″N 66°09′19″W	Venezuela
Mylossoma sp. (*sensu* Mateussi *et al.* in prep.)	LBP 2190	15518	Laguna des Castilleros, Orinoco basin	07°30′50″N 66°09′19″W	Venezuela
Ossubtus xinguense (Jégu 1992)	LBP 16563	64065	Rio Xingu, Amazon basin	03°15′24″S 52°05′47″W	Brazil
Piaractus mesopotamicus (Holmberg 1887)	LBP 4255	23804	Rio Paraná, upper Paraná basin	–	Brazil
Pristobrycon calmoni (Steindachner 1908)	LBP 2191	15554	Laguna des Castilleros, Orinoco basin	07°30′50″N 66°09′19″W	Venezuela
Pristobrycon striolatus (Steindachner 1908)	ANSP 188672	1631	Litanie rivier, Maroni basin	03°17′24″N 54°04′38″W	Suriname
Pristobrycon striolatus (Steindachner 1908)	ANSP 197515	10494	Rio Xingu, Amazon basin	02°51′55″S 51°59′21″W	Brazil
Pristobrycon striolatus (Steindachner 1908)	LBP 15050	61685	Rio Tapajós, Amazon basin	04°34′07″S 56°18′49″W	Brazil
Pristobrycon sp. (*sensu* Ota et al. 2013)	UFRO-ICT 7235	6010	Lago Cuniã, Madeira basin	08°19′56″S 63°29′00″W	Brazil
Pygocentrus cariba (Humboldt 1821)	LBP 10225	43107	Rio Apure, Orinoco basin	07°37′24″N 66°24′48″W	Venezuela
Pygocentrus nattereri Kner 1858	LBP 12693	43552	Rio Araguaia, Amazon basin	13°19′S 50°37′W	Brazil
Pygocentrus piraya (Cuvier 1819)	LBP 11336	45523	Lagoa da Tiririca, São Francisco basin	17°13′33″S 44°48′27″W	Brazil
Pygocentrus piraya (Cuvier 1819)	LBP 11286	48749	Rio São Francisco, São Francisco basin	09°56′46″S 37°06′15″W	Brazil
Pygocentrus piraya (Cuvier 1819)	LBP 11286	48750	Rio São Francisco, São Francisco basin	09°56′46″S 37°06′15″W	Brazil
Pygopristis denticulata (Cuvier 1819)	LBP 21609	62390	Rio Branco, Amazon basin	03°08′16″N 60°16′33″W	Brazil
Pygopristis denticulata (Cuvier 1819)	LBP 15529	63916	Rio Takutu, Amazon basin	03°17′57″N 59°55′38″W	Brazil
Serrasalmus altispinis (Merckx, Jégu & Santos 2000)	LBP 21606	61612	Rio Tapajós, Amazon basin	04°34′07″S 56°18′49″W	Brazil
Serrasalmus altuvei Ramírez 1965	LBP 22502	86574	Lago Yahuarcaca, Amazon basin	04°11′45″S 69°57′20″W	Colombia
Serrasalmus brandtii Lütken 1875	LBP 11269	48707	Rio São Francisco, São Francisco basin	09°51′23″S 37°06′30″W	Brazil
Serrasalmus compressus Jégu, Leão & Santos 1991	UFRO-ICT 24705	2546	Rio Jaciparaná, Madeira basin	09°16′58″S 64°23′53″W	Brazil
Serrasalmus eigenmanni Norman 1929	LBP 16210	66983	Rio Tracuá, Amazon basin	04°28′11″S 56°17′01″W	Brazil
Serrasalmus eigenmanni Norman 1929	LBP 20976	81155	Igarapé Carucaru, rio Jari, Amazon basin	00°56′00″S 52°32′30″W	Brazil
Serrasalmus elongatus Kner 1858	UFRO-ICT 1807	125	Lago do Cuniã, Madeira basin	08°20′36″S 63°30′48″W	Brazil
Serrasalmus elongatus Kner 1858	LBP 18025	72569	Igarapé Alencar, Amazon basin	03°05′57″S 58°27′18″W	Brazil
Serrasalmus gibbus Castelnau 1855	LBP 12811	41289	Rio Araguaia, Tocantins basin	13°18′37″S 50°36′47″W	Brazil
Serrasalmus hollandi Eigenmann 1915	UFRO-ICT 5631	6805	Igarapé Jatuarana, Madeira basin	08°46′25″S 64°02′49″W	Brazil
Serrasalmus humeralis Valenciennes 1850	LBP 15314	63320	Ribeirão Taquarussu, Tocantins basin	10°17′20″S 48°20′00″W	Brazil
Serrasalmus irritans Peters 1877	LBP 10229	43109	Rio Apure, Orinoco basin	07°37′24″N 66°24′48″W	Venezuela
Serrasalmus maculatus Kner 1858	LBP 3821	22107	Rio Negro, Paraguay basin	19°34′33″S 56°14′49″W	Brazil
Serrasalmus maculatus Kner 1858	LBP 3821	22108	Rio Negro, Paraguay basin	19°34′33″S 56°14′49″W	Brazil
Serrasalmus manueli (Fernández-Yépez & Ramírez 1967)	LBP 15032	61627	Rio Tapajós, Amazon basin	04°33′09″S 56°17′59″W	Brazil
Serrasalmus marginatus Valenciennes 1837	LBP 19855	79019	Lagoa do Guaraná, rio Baiá, upper Paraná basin	22°43′16″S 53°18′09″W	Brazil
Serrasalmus nigricans Spix & Agassiz 1829	LBP 4221	22719	Rio Juruá, Amazon basin	07°09′49″S 73°43′29″W	Brazil
Serrasalmus odyssei Hubert & Renno 2010	UFRO-ICT 20212	10000	Rio Pacaás-novos, Madeira basin	10°51′47″S 65°16′21″W	Brazil
Serrasalmus rhombeus (Linnaeus 1766)	LBP 15365	62022	Rio Culuene, Amazon basin	13°31′02″S 53°04′41″W	Brazil
Serrasalmus sp. “2n = 58” (*sensu* Ota et al. 2013)	LBP 14239	59423	Igarapé Montanha, Amazon basin	04°55′58″S 56°51′51″W	Brazil
Serrasalmus sp. “anal borda preta” (*sensu* Ota et al. 2013)	UFRO-ICT 17954	8091	Rio Karipuna, Madeira basin	09°11′30″S 64°37′21″W	Brazil
Tometes camunani Andrade, Giarrizzo & Jégu 2013	LBP 5196	26788	Rio Iratapuru, Amazon basin	00°34′03″S 52°34′41″W	Brazil

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Table 1 (*continued*)

Taxon	Museum number	Voucher number	Locality, basin	Coordinates	Country
Tometes camunani Andrade, Giarrizzo & Jégu 2013	LBP 20460	80790	Rio Iratapuru, Amazon basin	00°34′05″S 52°34′41″W	Brazil
Tometes kranponhah Andrade, Jégu & Giarrizzo 2016	ANSP 200868	13586	Rio Xingu, Amazon basin	03°35′00″S 51°49′24″W	Brazil
Tometes lebaili Jégu, Keith & Belmont-Jégu 2002	ANSP 188681	1634	Litanie rivier, Maroni basin	03°17′24″N 54°04′38″W	Suriname
Tometes trilobatus Valenciennes 1850	LBP 21012	81965	Rio Calçoene, Amazon basin	02°31′08″N 51°00′52″W	Brazil
Utiaritichthys longidorsalis Jégu, de Morais & Santos 1992	UFRO-ICT 19099	9141	Rio Roosevelt, Amazon basin	07°50′59″S 60°57′51″W	Brazil
Utiaritichthys sennaebragai Miranda Ribeiro 1937	MCP 44061	1	Rio Juruena, Amazon basin	13°41′00″S 59°00′00″W	Brazil
Alestes inferus Stiassny, Schelly & Mamonekene 2009	AMNH 242137	333238	Mpozo river, Congo basin	05°50′05.45S 13°29′42.13E	Democratic Republic of Congo
Anodus elongatus Agassiz 1829	LBP 4244	22720	Rio Juruá, Amazon basin	07°09′49″S 73°43′29″W	Brazil
Apareiodon ibitiensis Amaral Campos 1944	LBP 2890	18635	Rio Grande, Paraná basin	21°19′37″S 47°14′19″W	Brazil
Curimata vittata (Kner 1858)	LBP 13846	57302	Rio Tapajós, Amazon basin	04°16′49″S 59°59′26″	Brazil
Cynodon sp.	LBP 10227	43105	Rio Apure, Orinoco basin	07°37′24″N 66°24′48″W	Venezuela
Erythrinus erythrinus (Bloch & Schneider 1801)	LBP 6625	31955	Rio Paraná, Paraná basin	22°39′01″S 53°04′43″W	Brazil
Hemiodus quadrimaculatus Pellegrin 1909	LBP 21151	82973	Rio Amazonas, Amazon basin	03°04′49″N 51°28′50″W	Brazil
Hepsetus cuvieri (Castelnau 1861)	AMNH 242489	35–3404	Lac Nkolentulu, Mai Ndombe	01°33′38.7″S 18°42′43.1″E	Democratic Republic of Congo
Hoplias aimara (Valenciennes 1847)	LBP 20412	80691	Igarapé Pacanari, Jari basin	00°41′10″S 52°36′11″W	Brazil
Laemolyta garmani (Borodin 1931)	OS 18777	PE10-089	Rio Nanay, Amazon basin	03°45′06.0″S 73°17′14″W	Peru
Leporellus vittatus (Valenciennes 1850)	ANSP 182609	P6322	Rio Nanay, Amazon basin	03°42′49″S 73°16′43″W	Peru
Parodon hilarii Reinhardt 1867	LBP 10408	48929	Córrego Joaninha, São Francisco basin	17°19′32″S 44°46′01″W	Brazil
Prochilodus lineatus (Valenciennes 1837)	Uncatalogued	1	Rio Mogi-Guaçu, Paraná basin	–	Brazil
Rhaphiodon vulpinus Spix & Agassiz 1829	LBP 12660	43557	Rio Araguaia, Amazon basin	13°19′S 50°37′W	Brazil
Semaprochilodus kneri (Pellegrin 1909)	ANSP 187277	P4298	Río Apure, Orinoco basin	03°42′10″S 66°57′40″W	Venezuela
Steindachnerina argentea (Gill 1858)	STRI-4270	BFD01625	Turure River	10°35′22.9″S 61°05′25.2″W	Trinidad and Tobago

2. Material and methods

2.1. Taxon sampling and library preparation

Tissues of 69 species of serrasalmids were obtained (Table 1), including all genera. Outgroup taxa included related characiform families Alestidae, Anostomidae, Chilodontidae, Curimatidae, Cynodontidae, Erythrinidae, Hemiodontidae, Parodontidae, and Prochilodontidae (Table 1) according to previous phylogenetic hypotheses (Oliveira et al., 2011; Arcila et al., 2017). Tissue samples and voucher specimens are deposited at the Academy of Natural Sciences, Philadelphia (ANSP), Coleção de Peixes do Museu de Ciências e Tecnologia da PUCRS, Porto Alegre (MCP), Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul, Campo Grande (ZUFMS-PIS), Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista, Botucatu (LBP), Oregon State University, Corvallis (OS), Smithsonian Tropical Research Institute, Panama (STRI), and Universidade Federal de Rondônia, Porto Velho (UFRO-ICT).

Whole genomic DNA was extracted from ethanol-preserved muscle samples with the DNeasy Tissue Kit (Qiagen) and quantified using the Qubit dsDNA broad range Assay Kit (Invitrogen, Life Technologies) following manufacturer's protocol. The probe-set was developed for ostariophysan fishes to generate sequence data for 2708 UCE loci (Faircloth et al., 2020). Library preparation, sequencing, and raw data pipelining were performed by Arbor Biosciences® (Ann Arbor, Michigan, USA; http://www.arborbiosci.com) using the following protocol: DNA library preparation by modifying the Nextera (Epicentre Biotechnologies) library preparation protocol for solution-based target enrichment following (Faircloth et al., 2012) and increasing the number of PCR cycles following the tagmentation reaction to 20 as recommended by (Faircloth et al., 2012). Nextera library was used for preparation of the protocol of *in vitro* transposition followed by PCR to prune the DNA and attach sequencing adapters (Adey et al., 2010), and the Epicentre Nextera kit was used to prepare transposase-mediated libraries with insert sizes averaging 100 bp (95% CI: 45 bp) (Adey et al., 2010).

To prepare libraries, whole genomic DNA (concentration of 40 ng/μl) was first sheared with a QSonica Q800R instrument and selected to modal lengths of approximately 500nt using a dual-step SPRI bead cleanup. The DNA was converted to Illumina sequencing libraries with a slightly modified version of the NEBNext(R) Ultra(TM) DNA Library Prep Kit for Illumina(R). After connection of sequencing primers, libraries were amplified using KAPA HiFi HotStart ReadyMix (Kapa Biosystems) for six cycles using the manufacturer's recommended thermal profile and dual P5 and P7 indexed primers. After purification with SPRI beads, libraries were quantified with the Quant-iT(TM) Picogreen(R) dsDNA Assay kit (ThermoFisher). Pools were enriched comprising 100 ng each of 8 libraries (800 ng total) using the MYbaits (R) Target Enrichment system (MYcroarray) following manual version 3.0. After capture cleanup, the bead-bound library was resuspended in

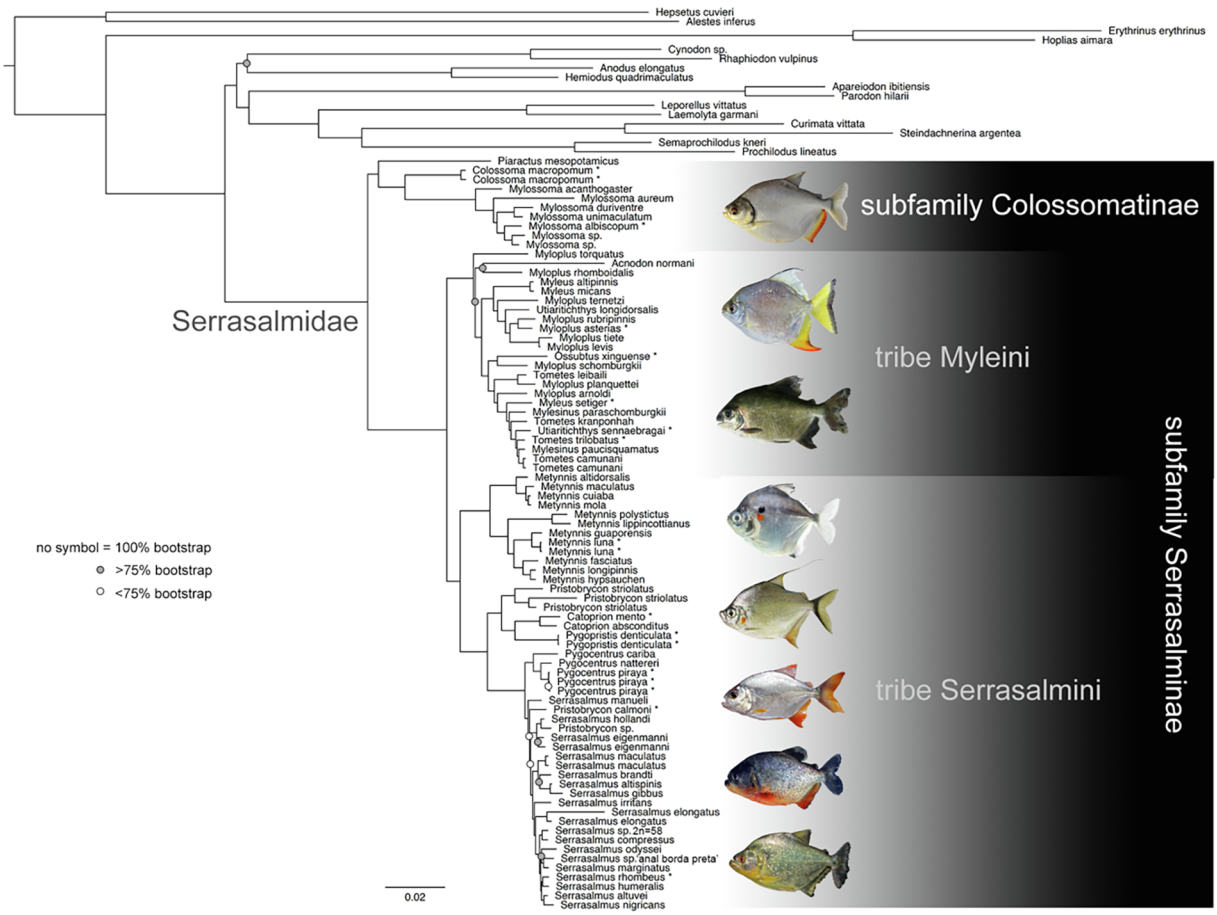

Fig. 2. Phylogenetic relationships of Serrasalmidae based on a maximum likelihood analysis of the 75% complete matrix of ultraconserved elements. All nodes have bootstrap values equal to 100% except where indicated. Asterisks denote type species. Fish photographs from top to bottom: *Mylossoma acanthogaster* (Mark Sabaj), *Myloplus arnoldi* (Mark Sabaj), *Mylesinus paraschomburgkii* (Mark Sabaj), *Metynnis luna* (Leandro Sousa), *Catoprion mento* (Claudio Oliveira), *Pygopristis denticulata* (Bruno Melo), *Pygocentrus nattereri* (Mark Sabaj), and *Serrasalmus rhombeus* (Rafaela Ota).

the recommended solution and amplified for 10 cycles using a universal P5/P7 primer pair and KAPA HiFi reagents. After purification, each captured library pool was quantified with PicoGreen and combined with all other pools in projected equimolar ratios prior to sequencing. Sequencing was performed across two Illumina HiSeq paired-end 100 bp lanes using v4 chemistry.

2.2. Raw data analysis

After sequencing, adapter contamination, low quality bases and the sequences containing ambiguous base calls were trimmed using the Illumiprocessor (github.com/faircloth-lab/illumiprocessor) within Phyluce (Faircloth, 2016). After trimming, Illumina reads were assembled into contigs on a species-by-species basis using ABySS (Simpson et al., 2009) (phyluce_assembly_assemblo_abyss). After sequence assembly, a custom Python program (match_contigs_to_probes.py) available at the Phyluce (Faircloth, 2016) was used, integrating LASTZ (Harris, 2007) to align species-specific contigs to our probe-UCE set (Faircloth et al., 2020). This last program creates a relational database of matches to UCE loci by taxon. Then, the get_match_counts.py command (also included in Phyluce) was used to query the database and generate fasta files for UCE loci that are identified across all taxa. A custom Python program (seqcap_align_2.py) was used to align contigs using the MAFFT algorithm (Katoh et al., 2002) and to perform edge trimming (phyluce_align_seqcap_align). Locus names from sequences lines were removed to ensure the sequence data aligned together are from the same loci (phyluce_align_remove_locus_name_from_nexus_lines). Phylogenetic analysis were performed with varying amounts of data (phyluce_align_get_only_loci_with_min_taxa) (50%, 75% and 90% of UCEs that are presented in the completed alignment matrices) to explore the potentially strong effect of missing data on phylogenetic reconstruction (Hosner et al., 2016; Streicher et al., 2016). Finally, in order to prepare alignment data for analysis, a concatenated phylip file was created (phyluce_align_format_nexus_files_for_raxml). Newly-generated UCE sequences are available at NCBI Sequence Read Archive submission under the code PRJNA603907 (SAMN13944248 – SAMN13944312).

2.3. Phylogenetic analyses

A maximum likelihood analysis (ML), a Bayesian inference (BI), and a species tree (AS) analysis were performed for the 50%, 75% and 90% complementary matrices. All trees were rooted in the clade *Alestes inferus* + *Hepsetus cuvieri*. For ML and BI analyses, the data was partitioned to account for variation in rates and patterns of molecular evolution among sites using PartitionUCE (Tagliacollo and Lanfear, 2018). The ML analysis of the concatenated alignment was performed in RAxML v8 (Stamatakis, 2014) using the autoMRE function for the extended majority-rule consensus tree criterion to access bootstrap support for individual nodes. This option allows the bootstrap convergence to determine if pseudoreplicates reach sufficient stable support values (Pattengale et al., 2009). The best tree search was performed under the parameter –N = 20, which specifies the number of alternative runs on distinct parsimony starting trees.

The BI of the concatenated alignment was performed using ExaBayes v1.4 (Aberer et al., 2014) with two independent runs (two chains each) of 10,000,000 generations each (stopped when converged) for the concatenated matrix using the GTR + G model, for different complementary matrices and for trimmed-alignment matrices. Tree space was sampled every 500 generations. Tracer v 1.6 (Rambaut and Drummond, 2009) was used to visualize the log of posterior probability within and between independent runs, to ensure that the average standard deviation of split frequencies was < 1%, the effective sample size (ESS) score > 200, and the potential scale reduction factor for estimated parameters approximately 1.0. The 50% most credible set of trees was generated from the posterior distribution of possible topologies using TreeAnnotator (burn-in: 10%). The AS was inferred from individual gene trees using ASTRAL-III (Zhang et al., 2018a). The individual gene trees used as input to ASTRAL-III were estimated by a ML analysis using a RaxML bootstrapped using the parameter –N = 5 and GTR-GAMMA model. Then, ASTRAL-III was used to estimate species trees from the best gene trees, and to reconstruct the majority-rule consensus tree of the results.

3. Results

Following sequencing and cleaning data, we obtained an average of 4,364,253 reads per sample from 97 specimens with a total of 2.27E +09 bp (Table S1). The three matrices with 50% complete (1,915 loci; 1,402,303 bp), 75% complete (1,553 loci; 1,200,606 bp), and 90% complete (500 loci; 370,867 bp) produced nearly identical topologies and strong node support for the three methods used (*i.e.* ML, BI and AS). The phylogeny in Fig. 2 corresponds to the results of ML tree for the 75% complete matrix. All trees are available as supplementary material (Figs. S1–S9). Most nodes of the ML tree presented 100% bootstrap support (Figs. 2, S1–S3), except the position of *Tometes* and some subclades of *Pygocentrus* and *Serrasalmus* (Figs. S1–S3). All nodes in the BI analysis received posterior probabilities equal 1.0 in the 50% complete matrix and a few lower values appeared in the other matrices for subclades of *Serrasalmus* and the position of *Pristobrycon* (Figs. S4–S6). AS analyses also showed high local posterior probabilities on most nodes, but with lower values in the clades involving *Acnodon*, *Myloplus*, *Tometes*, *Mylesinus*, *Utiaritichthys*, *Pristobrycon*, a subclade of *Pygocentrus* and several of *Serrasalmus* (Fig. S7–S9). Values equal or lower than 99% of bootstrap for ML and values lower than 0.99 for posterior probabilities in the BI and AS analyses are indicated in the nodes in the trees (Figs. S1–S9). The single difference among trees is the placement of *Pristobrycon calmoni* between the ML and BI trees of the 75% complete matrix (Figs. S2 and S5).

The monophyly of Serrasalmidae and the main clades within it received 100% node support in all three analyses. Serrasalmidae is the sister-taxon to a major clade including Cynodontidae, Hemiodontidae, Parodontidae, Anostomidae, Curimatidae, and Chilodontidae; Erythrinidae is the early-diverging family sister to all those families (Fig. 2). Within Serrasalmidae, two major sister clades were recognized: Colossomatinae and Serrasalminae (see taxonomic details below). Colossomatinae is sister to the remaining genera and is composed by *Piaractus* sister to *Colossoma* and *Mylossoma*. Interspecific relationships received maximum statistical support in all analysis. All species of *Mylossoma* were included herein. *Mylossoma acanthogaster*, the only species from the west of the Andes, was obtained as sister to all *Mylossoma* species occurring at east of the Andes; *M. aureum* from the Amazon basin is sister to two subclades: one with *M. duriventre* and *M. unimaculatum*, and the other with *M. albiscopum* and *Mylossoma* sp., an undescribed species from the Orinoco basin (Fig. 2).

Serrasalminae contains two major clades, the tribes Myleini and Serrasalmini. Myleini is composed by seven genera: *Acnodon*, *Mylesinus*, *Myleus*, *Myloplus*, *Ossubtus*, *Tometes*, and *Utiaritichthys*, all together represented herein at the first time in a molecular phylogeny, with maximum support (ML = 100, BI = 1.0, AS = 1.0). All genera were obtained as non-monophyletic, except *Ossubtus*, which is monotypic, and *Acnodon*, since the genus was represented here solely by *A. normani*. *Ossubtus* is sister to *Myloplus schomburgkii,* and *Acnodon normani* sister either to *Myloplus rhomboidalis* (50–75% ML, 50–75% BI) or *Myloplus torquatus* (90% ML, 90% BI, 50–90% AS), with this latter clade sister to the remaining species (90% ML, 90% BI, 50–90% AS). The type species of *Myloplus* (=*M. asterias*) form a clade with *M. rubripinnis*, *M. levis*, *M. tiete*, *M. ternetzi*, and *U. longidorsalis*; five other *Myloplus* appear in other subclades (*M. torquatus*, *M. rhomboidalis*, *M. schomburgki*, *M. planquettei*, and *M. arnoldi*). The type of *Myleus* (=*M. setiger*) is sister to a big clade including species of *Mylesinus*, *Tometes*, and *Utiaritichthys*, including the types of the two latter genera (*T. trilobatus* and *U. sennaebragai*).

The clade Serrasalmini is composed by *Metynnis*, *Pristobrycon*, *Catoprion*, *Pygocentrus*, *Pygopristis* and *Serrasalmus*. Within the clade, *Metynnis* is monophyletic and sister to all other genera. The species of *Metynnis* were divided in two major clades, with *M. altidorsalis*, *M. cuiaba*, *M. maculatus*, and *M. mola* in one subclade, and remaining species in the other (Fig. 2). Our topology resolves *Catoprion* sister to *Pygopristis* and this clade sister to *Pristobrycon striolatus*. The monophyly of *Pygocentrus* was supported by maximum support in all three methods. *Pygocentrus* is sister to the non-monophyletic *Serrasalmus*, because *Pristobrycon calmoni* (type species of *Pristobrycon*) and *Pristobrycon* sp. "Madeira" (*sensu* Ota et al., 2013) are nested within the analyzed species of *Serrasalmus* (Fig. 2).

To better accommodate species into taxonomic categories representing monophyletic groups, we recognize herein two subfamilies and two tribes within Serrasalmidae. We also provide morphological diagnoses relative to each of these major clades. Family-group names follow van der Laan et al. (2014).

Colossomatinae Kolmann et al. 2020

Type genus: *Colossoma* Eigenmann and Kennedy 1903.
Diagnosis: Colossomatinae differs from Serrasalminae by the absence of pre-dorsal spine (Machado-Allison, 1982).
Composition: *Colossoma*, *Mylossoma* Eigenmann & Kennedy 1903, and *Piaractus* Eigenmann 1903.

Serrasalminae Bleeker 1859

Type genus: *Serrasalmus* Lacepède 1803.
Diagnosis: Serrasalminae differs from Colossomatinae by the presence of pre-dorsal spine (Machado-Allison, 1982).
Composition: The subfamily is represented by two tribes: Myleini and Serrasalmini.
Remarks: Eigenmann (1915) defines the subfamily Serrasalminae to include the serrasalmids with "a single series of notched or lobate teeth", i.e. *Pygopristis*, *Gastropristis* (synonym of *Pygocentrus*), *Pygocentrus*, *Rooseveltiella* (synonym of *Pygocentrus*), *Pristobrycon* and *Serrasalmus*. Herein, species of Serrasalminae presents one or two series of teeth on the upper jaw, which may present variations, such as tri- or pentacuspidated, incisiform, and mamiliform.

Myleini Eigenmann 1903

Type genus: *Myleus* Müller & Troschel 1844.
Diagnosis: Pre-dorsal spine continuous to the first unbranched dorsal-fin ray (type I *sensu* Machado-Allison, 1982: character 29: 115); 18–30 branched dorsal-fin rays [except *Acnodon*, which presents 15–17 branched dorsal-fin rays, but it can be diagnosed by the lack of pre-pelvic abdominal spines (Nico et al., 2018)]; and six infraorbitals [except *Ossubtus*, which presents four (Andrade et al., 2016)].
Composition: *Acnodon* Eigenmann 1903, *Mylesinus* Valenciennes 1850, *Myleus* Müller & Troschel 1844, *Myloplus* Gill 1896, *Ossubtus*

Jégu 1992, *Tometes* Valenciennes 1850, and *Utiaritichthys* Miranda Ribeiro 1937.

Serrasalmini Bleeker 1859

Type genus: *Serrasalmus* Lacepède 1803.
Diagnosis: Pre-dorsal spine discontinuous to the first unbranched dorsal-fin ray (types II and III *sensu* Machado-Allison, 1982: characters 97 and 176: 151, 206); 13–17 branched dorsal-fin rays; five infraorbitals.
Composition: *Catoprion* Müller & Troschel 1844, †*Megapiranha* Cione, Dahdul, Lundberg & Machado-Allison 2009, *Metynnis* Cope 1878, *Pygocentrus* Müller & Troschel 1844, *Pygopristis* Müller & Troschel 1844, *Pristobrycon* Eigenmann 1915, and *Serrasalmus* Lacepède 1803.

4. Discussion

This study represents the first phylogenomic analysis including all genera of Serrasalmidae and the results support its monophyly and the position within the clade including Anostomoidea, Cynodontidae, Hemiodontidae, and Parodontidae. Previous phylogenies indicate Serrasalmidae either as sister to Hemiodontidae (Oliveira et al., 2011; Betancur et al., 2018; Burns and Sidlauskas, 2019), sister to Cynodontidae + Hemiodontidae (Arcila et al., 2017) or sister to Parodontidae, Hemiodontidae and Anostomoidea (Mirande, 2019). Although the exact position of the family within Characiformes is still uncertain, all studies agree with the monophyly of Serrasalmidae (Arcila et al., 2017; Betancur et al., 2018; Burns and Sidlauskas, 2019; Machado-Allison, 1983; Machado-Allison et al., 1989; Mirande, 2019; Oliveira et al., 2011; Ortí et al., 1996, 2008). The internal arrangement obtained here is also congruent with the most taxon-dense molecular studies focused exclusively on Serrasalmidae (Ortí et al., 1996, 2008; Thompson et al., 2014), including the recent exon-based phylogeny (Kolmann et al., 2020). Discrepancies include the presence of *Acnodon* as a fourth clade in the mtDNA studies (Ortí et al., 1996, 2008) and a morphology-based study (Cione et al., 2009), or *Piaractus* as the sister clade to all other three clades of serrasalmids (Betancur et al., 2018). Our results show consistently that *Acnodon* is one of the first lineages to diverge in Myleini and *Piaractus* is the sister of *Colossoma* + *Mylossoma* within Colossomatinae (Fig. 2) supporting previous phylogenies (Thompson et al., 2014; Kolmann et al., 2020).

4.1. Interspecific relationships in Colossomatinae

Colossomatinae is represented by three genera lacking a pre-dorsal spine. Uncertainties about the relationship among its genera are recurrent. Jégu (2004) identified *Colossoma* and *Piaractus* as the sister group to all other Serrasalmidae based on eleven synapomorphies (eight exclusives and three non-exclusives), as for example the presence of numerous dark, round spots on flanks (homoplastic in *Pristobrycon*, *Pygocentrus* and *Serrasalmus*) and five branchiostegal rays. Machado-Allison (1983) and Cione et al. (2009) recognized a different arrangement with *Mylossoma* sister to *Colossoma* and *Piaractus*. Herein, *Piaractus* appeared as sister to the clade composed by *Colossoma* and *Mylossoma*, corroborating previous molecular data (Kolmann et al., 2020; Ortí et al., 1996, 2008; Thompson et al., 2014).

A recent taxonomic revision of the species of *Mylossoma* occurring east of the Andes recognized four species (Mateussi et al., 2018) and their relationships are tested here for the first time. *Mylossoma acanthogaster*, endemic from the Lago Maracaibo at west of the Andes, is sister to all species distributed east of the Andes; this is a very distinctive species, with a very elongated body, already pointed out as a possible undescribed genus (Machado-Allison, pers. comm.). *Mylossoma aureum* resulted as sister to the major clade composed by *M. albiscopum* + *Mylossoma* sp. n and *M. duriventre* + *M. unimaculatum*, partially agreeing with a recent barcoding study of *Mylossoma* (Mateussi et al., 2017). Interestingly, *M. aureum* has the last abdominal spines clearly separated from the anal-fin origin, a unique feature within the genus (Mateussi et al., 2018). Both *Mylossoma duriventre* and *M. unimaculatum* are from the Brazilian Shield, while *M. albiscopum* and the undescribed species occur in lowlands of the Amazon and the Orinoco basin (Mateussi et al., 2018).

4.2. Subfamily Serrasalminae; tribe Myleini

We propose the subfamily Serrasalminae composed by both pacus and piranhas possessing a distinctive pre-dorsal spine. This clade was generally obtained in previous phylogenetic hypotheses (e.g. Cione et al., 2009; Kolmann et al., 2020; Ortí et al., 1996, 2008; Thompson et al., 2014), despite the different position of *Acnodon* in some of them (Cione et al. 2009; Ortí et al., 1996, 2008). The subfamily Serrasalminae is divided in two tribes: Myleini, represented by pacus, and Serrasalmini, represented by *Metynnis* and the piranhas.

Eigenmann (1915) proposed the subfamily Myleinae based on the presence of two series of premaxillary teeth and the presence of a conical symphyseal teeth behind the main series of dentary. Species of Myleini (former *Myleus* clade *sensu* Ortí et al. 2008) redefined herein differs of Eigenmann's Myleinae by the exclusion of *Mylossoma*, *Colossoma*, and *Piaractus* (herein defined as Colossomatinae), and *Metynnis* and *Catoprion*, which are closely related to piranhas of the tribe Serrasalmini. Myleini is characterized here by the presence of a pre-dorsal spine continuous to the first unbranched dorsal-fin ray (*sensu* Machado-Allison 1982; 1983). Additionally, all species present a series of characters related to sexual dimorphism: (1) mature males possess a bilobed anal fin with stiff hooks laterally curved on distalmost lepidotrichia of branched rays; (2) long and thin filaments on branched dorsal-fin rays and (3) more intense body colouration during breeding period. In contrast, females have very rigid unbranched anal-fin rays with first rays extremely elongated and forming a falcate margin, and more discrete body colouration (e.g. Jégu, 1992; Jégu and dos Santos, 1988; 1990; 2002; Jégu et al., 1992; Jégu, 2004). All these differences in general body shape and coloration confound the recognition of intra and interspecific limits of species in the tribe.

Therefore, Myleini represents a complex group, with the most controversial and problematic relationships in Serrasalmidae. Molecular studies had already evidenced the paraphyly of *Mylesinus*, *Myloplus*, and *Tometes* (Ortí et al., 2008, 1996; Thompson et al., 2014). None of these, together with *Myleus* and *Utiaritichthys*, were recognized as monophyletic here. The exon-based phylogeny found *Mylesinus* and *Utiaritichthys* as monophyletic groups, though the authors used only one species each (Kolmann et al., 2020). The other two genera are either monotypic (*Ossubtus*) or represented by a single species (*Acnodon*) in our phylogeny. Therefore, most of the genera within this tribe are paraphyletic and need further taxonomic review. Additionally, the recent DNA barcoding study highlighted the occurrence of multiple lineages in *Mylesinus*, *Myloplus*, and *Tometes* (Machado et al., 2018).

Acnodon has been placed in different portions in serrasalmid phylogenies (Fig. 1), either as sister to all remaining genera except *Colossoma*, *Mylossoma* and *Piaractus* (Cione et al., 2009; Ortí et al., 2008, 1996), or sister to all other Myleini genera (Freeman et al., 2007; Jégu, 2004; Kolmann et al., 2020; Thompson et al., 2014). Herein, we obtained *Acnodon normani* as sister to *Myloplus torquatus* or *M. rhomboidalis* depending on the analysis. As only *A. normani* could be used in this study, the position of the genus remains uncertain within Myleini.

Despite recent taxonomic efforts in describing the diversity and solving punctual taxonomic problems of many Myleini genera (e.g. Andrade et al. 2016 in *Tometes*, Andrade et al. 2018a in *Myloplus rubripinnis*, Andrade et al. 2019 in *Myleus pacu*), limits among genera and the inclusion of more species, especially of *Myleus*, might contribute to better clarify intergeneric and interspecific relationships within Myleini.

4.3. Tribe Serrasalmini

Serrasalmini (the former "piranha" clade *sensu* Ortí et al., 2008) is diagnosed here by the presence of a pre-dorsal spine not continuous to the first dorsal-fin ray (types II and III *sensu* Machado-Allison, 1982, 1983). Furthermore, as in Myleini, features related to sexual dimorphism can also be observed. In *Metynnis*, *Catoprion*, *Pristobrycon striolatus*, and *Pygopristis*, mature males present only one lobe at the anterior portion of the anal fin (*vs*. two lobes in Myleini) and more intense general color pattern during breeding periods, while females have falcate anal fin, but without the extreme prolongation of first rays observed in Myleini (e.g. Machado-Allison and Fink, 1996; Mateussi et al., 2020; Zarske and Géry, 1999). Based on the phylogeny, these features were apparently lost in the clade with *Serrasalmus* (including two species of *Pristobrycon*) and *Pygocentrus*.

All previous phylogenetic hypotheses for Serrasalmidae, both morphological and molecular, recognized *Metynnis* as the first clade to diversify within Serrasalmini and sister to all piranha genera (*i.e. Catoprion*, *Pristobrycon*, *Pygocentrus*, *Pygopristis*, and *Serrasalmus*) (Cione et al., 2009; Dahdul, 2007; Freeman et al., 2007; Jégu, 2004; Ortí et al., 1996, 2008; Thompson et al., 2014). Morphological synapomorphies for *Metynnis* include reduction on number of infraorbital bones and reduction of a section of the *adductor mandibularis* (Machado-Allison, 1983). Cione et al. (2009) obtained a paraphyletic *Metynnis*, while our analyses support its monophyly, including a higher number of representatives of the genus (11 of the 15 valid species). Species were divided in two major clades, similarly as proposed by Ota (2015), with the exception of *Metynnis lippincottianus* and *M. polystictus* as more closely related to *M. fasciatus*, *M. guaporensis*, *M. hypsauchen*, *M. longipinnis*, and *M. luna*.

The relationships between *Catoprion* and *Pygopristis* obtained here reject the hypotheses of *Catoprion* as sister to remaining piranhas (Ortí et al., 1996, 2008). However, it corroborates several previous studies in which *Catoprion* is sister to *Pygopristis* (Dahdul, 2007; Freeman et al., 2007; Hubert et al., 2007; Thompson et al., 2014) and also as sister to *Pristobrycon striolatus* (Freeman et al., 2007; Hubert et al., 2007; Thompson et al., 2014). The second species of *Catoprion* was recently described from the Amazon basin (Mateussi et al., 2020).

Previous studies using mtDNA (Freeman et al., 2007; Hubert et al., 2007; Ortí et al., 2008, 1996) returned both *Serrasalmus* and *Pygocentrus* as non-monophyletic genera. Thompson et al. (2014), using nuclear genes, obtained the monophyly of *Pygocentrus* and the paraphyly of *Serrasalmus* with *Pristobrycon calmoni* (type species of *Pristobrycon*) nested within *Serrasalmus* with high support. Relationships within *Pygocentrus* also confirm the arrangement of *P. cariba* as sister to *P. nattereri* and *P. piraya* obtained by the recent molecular study (Mateussi et al., 2019). Details for *Pristobrycon* are discussed below.

The knowledge about the diversity of Serrasalmidae increased significantly in the last decade, with the description of 14 species (e.g., Andrade et al. 2016, 2018a,b, 2019; Escobar et al. 2019; Mateussi et al., 2020; Ota et al. 2016, 2020), some of them revealed by molecular techniques using integrative taxonomy (Andrade et al., 2017; Escobar et al., 2019; Mateussi et al., 2020; Ota et al., 2020). This number is only inferior to those observed for Anostomidae, Characidae, Crenuchidae, and Curimatidae within the Characiformes (Fricke et al., 2020a). Machado et al. (2018) in a broad coverage DNA barcoding of Serrasalmidae, comprising more than one thousand specimens, identified an extraordinary underestimated diversity within the family. This hidden richness complicates the studies of phylogenetic relationships within Serrasalmidae, which are even more aggravated by morphological diagnosis for genera that are possibly not monophyletic, mainly those characters associated with the type and arrangement of premaxillary teeth (Jégu et al. 2004), likely a result of convergence related to feeding habits without phylogenetic signal, as for example the species with incisiform teeth associated to rheophilic environments (Andrade et al., 2013, 2016, 2017; Huie et al., 2019).

4.4. Systematics of Pristobrycon

Pristobrycon was described by Eigenmann (1915) to include five species: *P. aureus*, *P. calmoni* (type species), *P.* (*Serrasalmo*) *emarginatus*, *P. scapularis*, and *P. striolatus*. The genus was diagnosed by having "intermediate" features between *Serrasalmus* and *Rooseveltiella* Eigenmann, 1915 (=*Pygocentrus nattereri*), e.g. the ectopterygoid toothless or with few poorly developed teeth, snout short and flattened anteriorly, and infraorbitals well developed covering the cheeks. Machado-Allison and Fink (1996) discovered other two species from Venezuela: *P. careospinus* and *P. maculipinnis*. Posteriorly, *Serrasalmo emarginatus* was considered *species inquirenda* in *Serrasalmus*, and *P. scapularis* a junior synonym of *P. striolatus* (Jégu, 2003). Thus, five species of *Pristobrycon* are currently recognized: *P. aureus*, *P. calmoni*, *P. careospinus*, *P. maculipinnis*, and *P. striolatus*, distributed throughout the Amazon and Orinoco basins, and the Guianas (Fricke et al., 2020b).

The genus *Pristobrycon* was considered a junior synonym of *Serrasalmus* by Norman (1929), but later validated as a subgenus of *Serrasalmus* by Géry (1972). The validity and monophyly of *Pristobrycon* was contested in morphological proposes (Machado-Allison, 1982, 1983, 1985). Several osteological features were proposed to further diagnose the genus from the remaining piranha genera, as premaxillary and dentary teeth with lateral cusps well developed, neurocranium short, frontal, parietal and pterotic bones laterally expanded, and dorsal posttemporal fossa reduced (*vs*. the opposite states in *Serrasalmus* and *Pygocentrus*) (Machado-Allison, 1985).

Machado-Allison & Fink (1996) called *Pristobrycon* an "artificial genus" and divided it in two groups based on presence or absence of a pre-anal spine, and by the extrinsic musculature on the anterior gasbladder chamber. Among the five currently valid species, only *Pristobrycon calmoni* and *Pristobrycon* sp., which represents an undescribed species, possess pre-anal spine and the musculature in the gasbladder chamber. All previous molecular hypotheses identified *P. calmoni* nested within *Serrasalmus* while *P. striolatus* was recognized as sister to *Pygocentrus* and *Serrasalmus* (Dahdul, 2007), or sister to *Catoprion* and *Pygopristis* (Freeman et al., 2007; Hubert et al., 2007; Kolmann et al., 2020; Ortí et al., 1996, 2008; Thompson et al., 2014) as obtained herein.

The well-supported resolution placing *Pristobrycon calmoni* (type species of *Pristobrycon*) nested within *Serrasalmus* (including the type species *S. rhombeus*) suggests a reallocation of the species of *Pristobrycon* presenting a pre-anal spine (*P. calmoni* and *Pristobrycon* sp.) into *Serrasalmus*. However, in our phylogenetic hypothesis, *P. striolatus* is placed in another clade sister to *Catoprion*, suggesting that the three *Pristobrycon* species lacking the pre-anal spine (*P. careospinus*, *P. maculipinnis*, and *P. striolatus*) should be allocated in a new genus. In the absence of an available genus to allocate those three latter species, it is now premature to propose changes in the classification involving species of the polyphyletic *Pristobrycon*. The taxonomic review and phylogenetic relationships of *Pristobrycon* is being currently conducted by authors of this paper.

4.5. Perspectives on the systematics of Serrasalmidae

Taxonomic investigations in Serrasalmidae are still impaired by high levels of variation in body shape and color pattern presented by several Myleini and Serrasalmini genera during the development and breeding periods (Jégu, 2003; Ota et al., 2016). Studies integrating molecular and morphological data (e.g., Andrade et al. 2017; Mateussi et al. 2020; Ota et al., 2020) are essential to recognize and delimit species within the family, with many other genera needing attention (e.g., *Serrasalmus*). Furthermore, an interesting long-term approach is mapping morphological characters in the molecular phylogeny to reconstruct ancestral states and provide the evolutionary understanding of the processes involved in species diversification. Future directions in the systematics involve the investigation of the molecular diversity in

Colossomatinae (e.g, *Colossoma* and *Piaractus*) and *Metynnis*, and the systematic question related to *Pristobrycon*.

Besides several taxonomic inconsistencies in Serrasalmidae revealed by previous studies and confirmed herein, this study represents the most species-dense molecular phylogeny of the family using a robust method to provide relative resolution in shallow nodes. The results presented herein provide a strong foundation for the urgent taxonomic revision of some groups and highlight the necessity of integrative studies using morphological and molecular tools to address generic inconsistencies within the two tribes of Serrasalminae. Our phylogeny will additionally provide an interesting phylogenetic framework for further studies in the fields of biogeography and macroevolution.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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