Supplemental material for:

Phylogenomics of piranhas and pacus (Serrasalmidae) uncovers how dietary convergence and parallelism obfuscate traditional morphological taxonomy.

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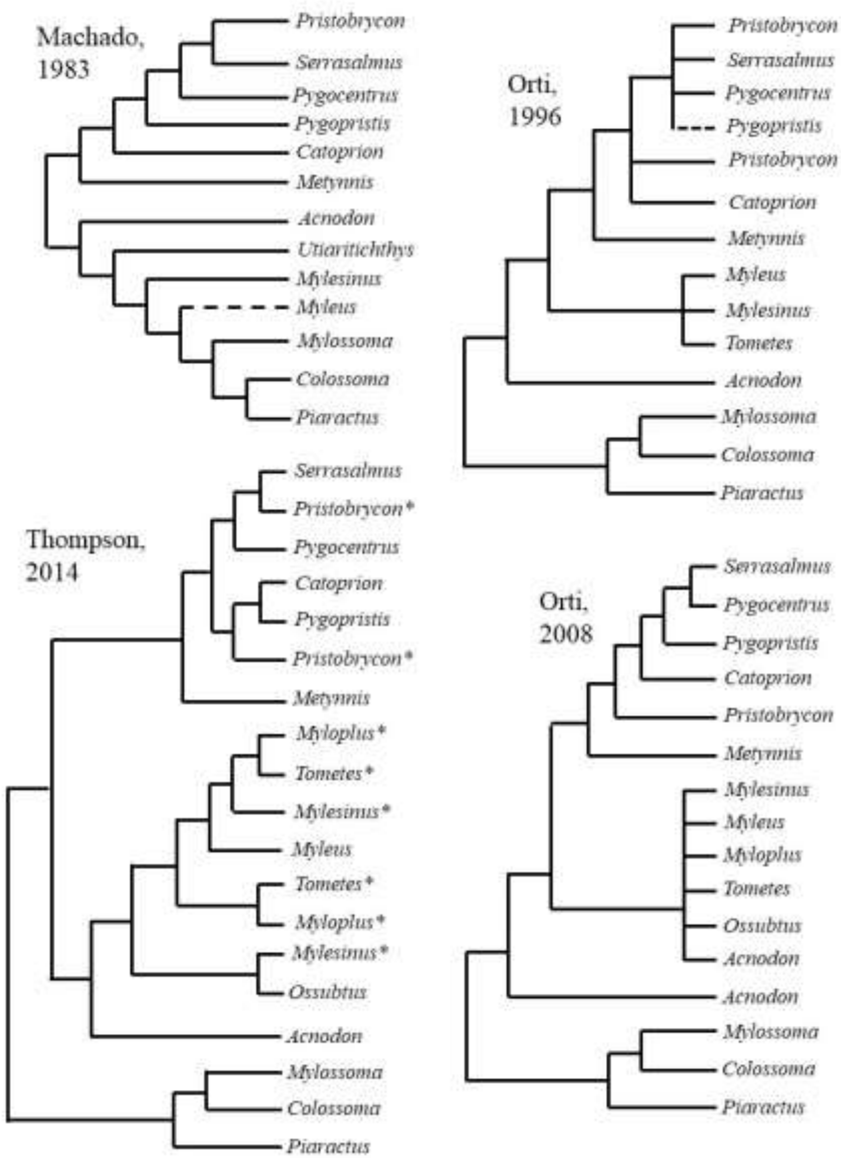
S1 - PREVIOUS MORPHOLOGICAL & MOLECULAR PHYLOGENETIC HYPOTHESES FOR SERRASALMIDAE
(reproduced and modified from their original figures)



**asterisks represent taxa found to be non-monophyletic in each's respective study. Dashed lines indicate the probably position of un-sampled taxa in each study.

S2 - DESCRIPTION & RATIONALE OF FOSSIL CALIBRATIONS

We used BEAST 2 (v2.5.0; Bouckaert et al., 2014) to generate relaxed-clock divergence time estimates (Drummond et al., 2006) on four 50-gene subsets of our data, randomly selected from the 200 most complete genes, and pruned so that only one tip per taxon remained. First, we converted the concatenated nucleotide phylogeny estimated from IQTree to a chronogram under penalized likelihood using the *chronos* function in R (ape v. 5.3; Paradis et al., 2019). This chronogram was used as a starting tree for the BEAST 2 analyses (Supplemental Files), the topology of the resulting trees was also constrained to match the concatenated nucleotide phylogeny. Each subset of 50 genes was run independently in BEAST 2 twice for 200,000,000 generations. All subsets had 103 included taxa, and Subset 1 had 4425 sites, Subset 2 had 5264 sites, Subset 3 had 5288 sites, and Subset 4 had 5096 sites. For each BEAST2 run, we used the GTR + gamma as our site model for each locus. We used a birth-death model tree prior for node time estimation, allowing for both speciation and extinction rates to vary for any given lineage (Drummond et al., 2006). We fixed the topology of our starting tree by turning off the following operators in BEAST2: (1) set 'wide-exchange' ="false", (2) set 'narrow-exchange' to "false", (3) set 'subtree-slide' to 0, and (4) set 'Wilson-Balding' to 0.

To explore how the ambiguity surrounding these fossils alters our estimates of serrasalmid diversification, we used two different fossil calibration schemes and contrast the timelines produced by these analyses (and by previous studies, e.g. Broughton et al., 2013; Burns & Sidlauskas, 2019). We calibrated Scheme 1 with 15 fossil calibrations and Scheme 2 with 14 fossils. We used exponential distributions on each fossil prior except for the root, which used a normal distribution (Chen et al., 2010), in order to account for increasing uncertainty at further points in the past. Mean and standard deviations were estimated based on the calibration setting from other studies (e.g. Broughton et al., 2013; Chen et al., 2010; Thompson et al., 2014; Burns & Sidlauskas, 2019) which used the same fossils as calibrations points.

The first eleven fossil calibrations dealt with calibrations external to Serrasalmidae, in other characiform families. Within Characoidea, we dated the divergence between Characidae and Chalceidae, using fossil *Paleotetra* from the Aiuruoca Tertiary Basin (Weiss et al., 2012, 2014), Minas Gerais State in Eocene-Oligocene sediments (Garcia et al., 2000) (minimum age/offset = 23.0 mya, mean = XX). Two fossils were used to date within Alestoidea; for dating the base of Alestoidea *sans* Hepsetidae, we used fossil †*Alestoides eocaenicus* from Eocene Dormaal, near Brabant, Belgium (minimum age/offset = 48.6 mya, mean = 3.2) (Zanata & Vari, 2005; Gaudant & Smith, 2008; Chen et al., 2013). We also used fossils of the extant genus *Hydrocynus* to date the divergence between *Hydrocynus* + *Micralestes*, from the middle Eocene Hamada of Méridja deposits, in southwestern Algeria (Hammouda et al., 2016) (minimum age = 37.0 mya/offset, mean = 3.85).

We used two fossils pertaining to Erythrinidae; firstly, we used fossils attributed to Erythrinoidea (Gayet et al., 2003) from the Late Cretaceous to Paleocene of Bolivia (Gayet & Brito, 1989; Gayet, 1991; Gayet and Meunier, 1998) to date the root of our phylogeny, i.e. the node uniting Characoidea with Curimatoidea + Alestoidea (*sensu* Betancur et al., 2019) (minimum age/offset = 58.2 mya, mean = 13.82). To calibrate the node uniting *Hoplerythrinus* + *Hoplias*, we used teeth attributed to †*Paleohoplias assisbrasiliensis* (Gayet et al., 2003) from the late Miocene Solimões Formation of Acre State, Brazil (Latrubesse et al., 1997; Cione et al., 2003; Grosse et al., 2011) (minimum age = 7.2 mya/offset, mean = 17.0). Finally, we used fossil cynodontid teeth to calibrate the node uniting *Hydrolycus* + [*Rhaphiodon*, *Cynodon*]. These fossils are from middle Miocene sediments associated with the La Venta fauna near Tolima, Colombia (minimum age/offset = 7.2 mya, mean = 17.0; Lundberg, 1997; Cione & Casciotta, 2010).

Three fossils were used to date within anostomoids and one fossil from related Parodontidae; for dating the base of Anostomidae, we used a fossil oral tooth attributed to *Leporinus* sp. from the lower Pozo Formation, Contamana, Peru (Antoine et al. 2016), Middle Eocene sediments (minimum age/offset = 35.0 mya, mean = 7.7; Burns & Sidlauskas, 2019). We also used fossils of †*Leporinus scalabrinii* (Bogan et al., 2012) to date the divergence between *Abramites hypselonotus* + *Leporinus striatus*, from the late Miocene deposits of the Ituzaingó Formation in Entre Ríos, Argentina (Marshall et al., 1983; Cione et al., 2000, 2009) (minimum age/offset = 6 mya, mean = 9.7). Finally, to calibrate the node uniting *Cyphocharax* + *Psectrogaster* with *Curimata*, we used †*Cyphocharax mosesi* from the Tremembe Formation, Sao Paulo, Brazil in Oligocene sediments (Malabarba, 1996) (minimum age = 23.0 mya/offset, mean = 11.7). †*Cyphocharax mosesi* was originally proposed as forming a polytomy with the genera *Cyphocharax*, *Curimatella*, and *Steindachnerina* (Malabarba, 1996; Burns & Sidlauskas, 2019).

We also used fossil teeth attributed to *Parodon* by Roberts (1975) to date the divergence between *Apareiodon* + *Parodon*, from mid-late Miocene deposits of the Loyola Formation near Cuenca, Ecuador (Bristow, 1973) (minimum age/offset = 11.2 mya, mean = 15.7) (Hungerbühler et al 2002).

Within Serrasalmidae, four fossil calibrations were used; firstly, for Scheme 2, we used the isolated pacu teeth first described in Gayet (1991), and used by Broughton et al. (2013) and Thompson et al. (2014) to date the divergence of serrasalmids from other non-serrasalmid characiforms (minimum age = 61.0 mya/offset, mean = 12.9). Whereas Broughton et al. used this fossil to represent the MRCA for *Pygocentrus* + *Hemiodus*, Thompson et al. used these fossil teeth to calibrate the node uniting *Serrasalmus* + *Piaractus*. For Scheme 1, we removed this calibration and replaced with pacu teeth described by DeCelles & Horton (2003) from the Paleocene-Eocene Santa Luca Formation, Bolivia (minimum age = 38.0 mya/offset, mean = 6.75). To calibrate the node uniting *Colossoma* + *Mylossoma*, we used teeth and partially articulated jaws documented by Lundberg et al. (1986) and Dahdul (2004) from the Miocene Castillo Formation, Venezuela (Rincon et al., 2014) (minimum age/offset = 17.2 mya, mean = 7.0). The pacu fossils from above predate fossils of *Piaractus* (Sanchez-Villagra & Aguilera, 2006) from the Tortonian Urumaco Formation in Falcón State, Venezuela (Dahdul, 2004). Next, we used fossil teeth attributed to indeterminate myleines (medium-sized pacus) to calibrate the MRCA of *Acnodon* + *Myloplus* (Roberts, 1975; Dahdul, 2004) from the mid-late Miocene Loyola Formation near Cuenca, Ecuador (Bristow, 1973) (minimum age/offset = 11.2 mya, mean = 9.0) (Hungerbühler et al 2002; Dahdul, 2004). Finally, to calibrate the MRCA of all piranha genera, we used the upper Miocene fossil premaxilla described as †*Megapiranha paranensis* discovered in Entre Ríos, Argentina (Cione et al., 2009) (minimum age/offset = 6.8 mya, mean = 10.4).

Convergence of each gene subset was assessed individually in Tracer (v. 1.7.1) by checking that ESS values were greater than 200 for all parameters. Independent runs from each of the four different subsets were combined in LogCombiner if their 95% highest posterior densities for divergence times overlapped, and a maximum clade credibility tree was generated in TreeAnnotator for each of the two calibration schemes.

S3 - TIME CALIBRATION, GEOLOGICAL DATING, & FOSSIL REFERENCES

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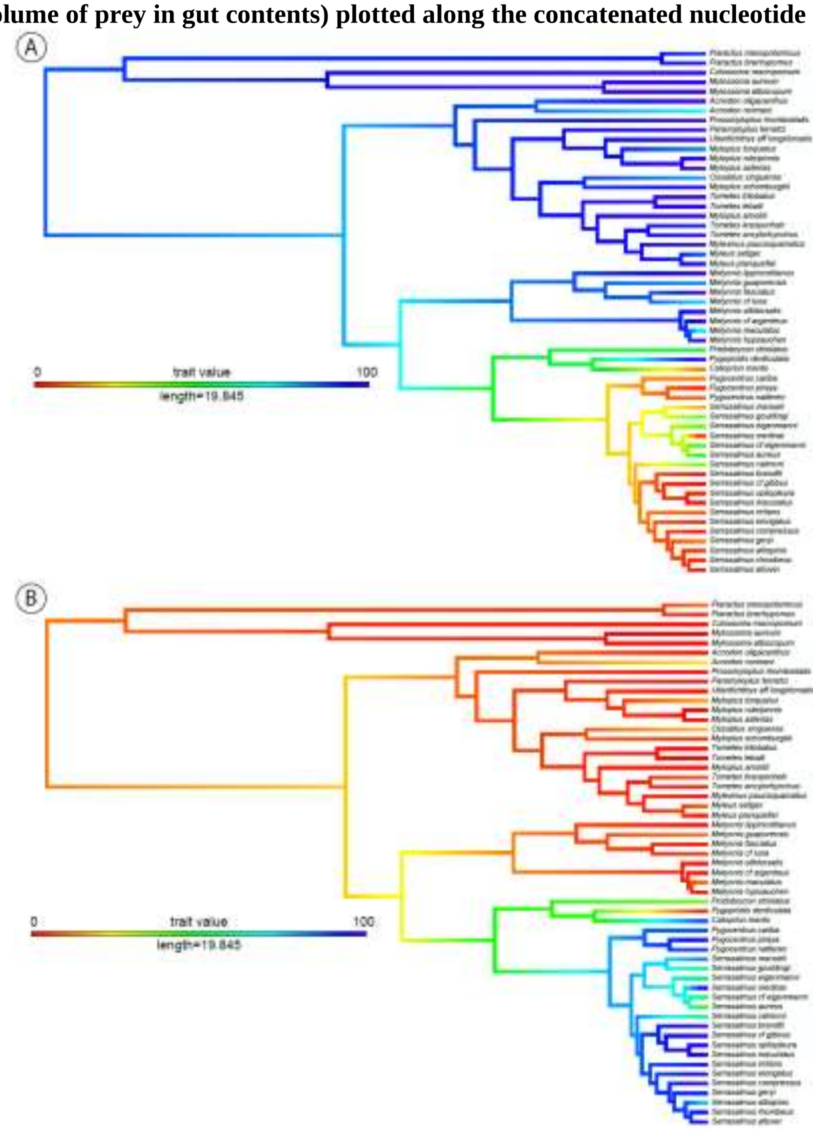
S4 - TAXON & DIET MATCHING TABLE

Species	Diet Category	Reference(s)
Acnodon normani	folivore	Leite & Jégu 1990; Andrade et al., 2019a
Acnodon oligacanthus	folivore*	Planquette et al., 1996; Mol, 2012
Catoprion mento	fins.scales	Vieira & Gery, 1979; Nico & Taphorn, 1988; Nico & Morales, 1994; Wantzen et a
Colossoma macropomum	fruits.seeds	Goulding, 1980; Goulding & Carvalho, 1982; Lucas, 2008
Metynnis altidorsalis	planktivore	Mol, 2012; do Carmo, 2013; Ota, 2015
Metynnis argenteus	planktivore	do Carmo, 2013
Metynnis fasciatus	folivore	Ota, 2015
Metynnis guaporensis	folivore	Andrade et al., 2019; Ota, 2015
Metynnis hypsauchen	planktivore	Araujo-Lima et al., 1986; Ota, 2015
Metynnis lippincottianus	planktivore	Canan & Gurgel 2002 (as *M. roosevelti*); Ramos et al., 2008
Metynnis luna	planktivore	Ota, 2015; Andrade et al., 2019
Metynnis maculatus	planktivore	Silva-Camacho et al., 2014; Pelicice & Agostinho, 2006
Mylesinus paucisquamatus	folivore	Santos et al., 1997; Dary et al., 2017
Myleus setiger	folivore	Dary et al., 2017; Andrade et al., 2019
Myloplus arnoldi	folivore	Zuluaga-Gómez et al. 2016
Myloplus asterias	fruits.seeds	Nico, 1991; Dary et al 2017; Andrade et al., 2019
Myloplus planquettei	fruits.seeds	Jegu et al., 2003
Myloplus rhomboidalis	folivore	Boujard et al., 1990; Andrade et al., 2019
Myloplus rubripinnis	folivore	Dary et al., 2017; Gonzalez & Vispo, 2002; Andrade et al., 2019
Myloplus schomburgkii	folivore	Zuluaga-Gómez et al., 2016; Dary et al., 2017; Andrade et al., 2019
Myloplus ternetzi	folivore	Boujard et al., 1990; Merona et al., 2008
Myloplus torquatus	folivore	Nico, 1991; Dary et al., 2017
Mylossoma aureum	fruits.seeds	Soares et al. 1986; Dos Santos 1990; Pouilly et al., 2003, 2004
Mylossoma albiscopum	folivore	Gonzalez & Vispo, 2003; Pouilly et al., 2003, 2004
Ossubtus xinguense	folivore*	Jegu, 1992; Andrade et al., 2016b
Piaractus brachypomus	fruits.seeds	Goulding, 1980; Lucas, 2008
Piaractus mesopotamicus	fruits.seeds	Galetti et al., 2008; Sório et al., 2014
Pristobrycon aureus	fruits.seeds	Goulding, 1980
Pristobrycon calmoni	piscivory	Gonzalez & Vispo, 2002 (as *Pristobrycon* spp); Nico, 1991
Pristobrycon striolatus	piscivory	Goulding, 1988; Nico & Taphorn, 1988; Nico, 1991
Pygocentrus cariba	piscivory	Gonzalez & Vispo, 2002; Nico & Taphorn, 1988; Winemiller, 1989
Pygocentrus nattereri	piscivory	Nico & Taphorn, 1988; Ferreira et al 2014
Pygocentrus piraya	fins.scales	Trindade & Jucá-Chagas, 2008
Pygopristis denticulata	fruits.seeds	Nico, 1991 (juveniles); Nico & Taphorn, 1998
Serrasalmus altispinis	piscivory	Andrade et al., 2019
Serrasalmus altuvei	piscivory	Nico, 1991; Nico & Taphorn, 1998

Serrasalmus brandtii	piscivory	Pompeu, 1999; Gurgel et al., 2002; Trindade & Jucá-Chagas, 2008
Serrasalmus compressus	fins.scales	Pouilly et al., 2003, 2004
Serrasalmus eigenmanni	piscivory	Merona et al., 2001; Pouilly et al 2003; Dary et al., 2017
Serrasalmus elongatus	fins.scales	Nico & Taphorn, 1998; Röpke et al., 2014
Serrasalmus geryi	fins.scales	Araujo-Lima et al., 1995; do Carmo, 2013
Serrasalmus gouldingi	fins.scales	Prudente et al., 2016
Serrasalmus irritans	piscivory	Nico & Taphorn, 1988
Serrasalmus maculatus	piscivory	Carvalho et al., 2007; Behr & Signor, 2008
Serrasalmus manueli	piscivory	Dary et al 2017; Nico, 1991
Serrasalmus medinai	fins.scales	Winemiller, 1989; Nico, 1991
Serrasalmus rhombeus	piscivory	Nico & Taphorn, 1988; Gonzalez & Vispo, 2002; Pouilly et al., 2003
Serrasalmus spilopleura	piscivory	Wantzen et al., 2002; Raposo & Gurgel, 2003
Tometes ancylorhynchus	folivore*	Andrade et al., 2016, 2019
Tometes kranponhah	folivore*	Andrade et al., 2016, 2018, 2019
Tometes lebaili	folivore*	Mol, 2012; Jégu et al., 2002a
Tometes trilobatus	folivore*	Mol, 2012; Jégu et al., 2002b
Utiaritichthys aff longidorsalis	folivore*	Jegu etal., 1989; Pereira & Castro, 2014

*feeds predominantly on Podostemaceae, to the exclusion of other types of plants

Continuous diet data (%volume of prey in gut contents) plotted along the concatenated nucleotide phylogeny



(A) % volume plant (seeds, fruits, leaves, stems, algae) materials in diet

(B) % volume fish (flesh, scales, fins, bones) materials in diet

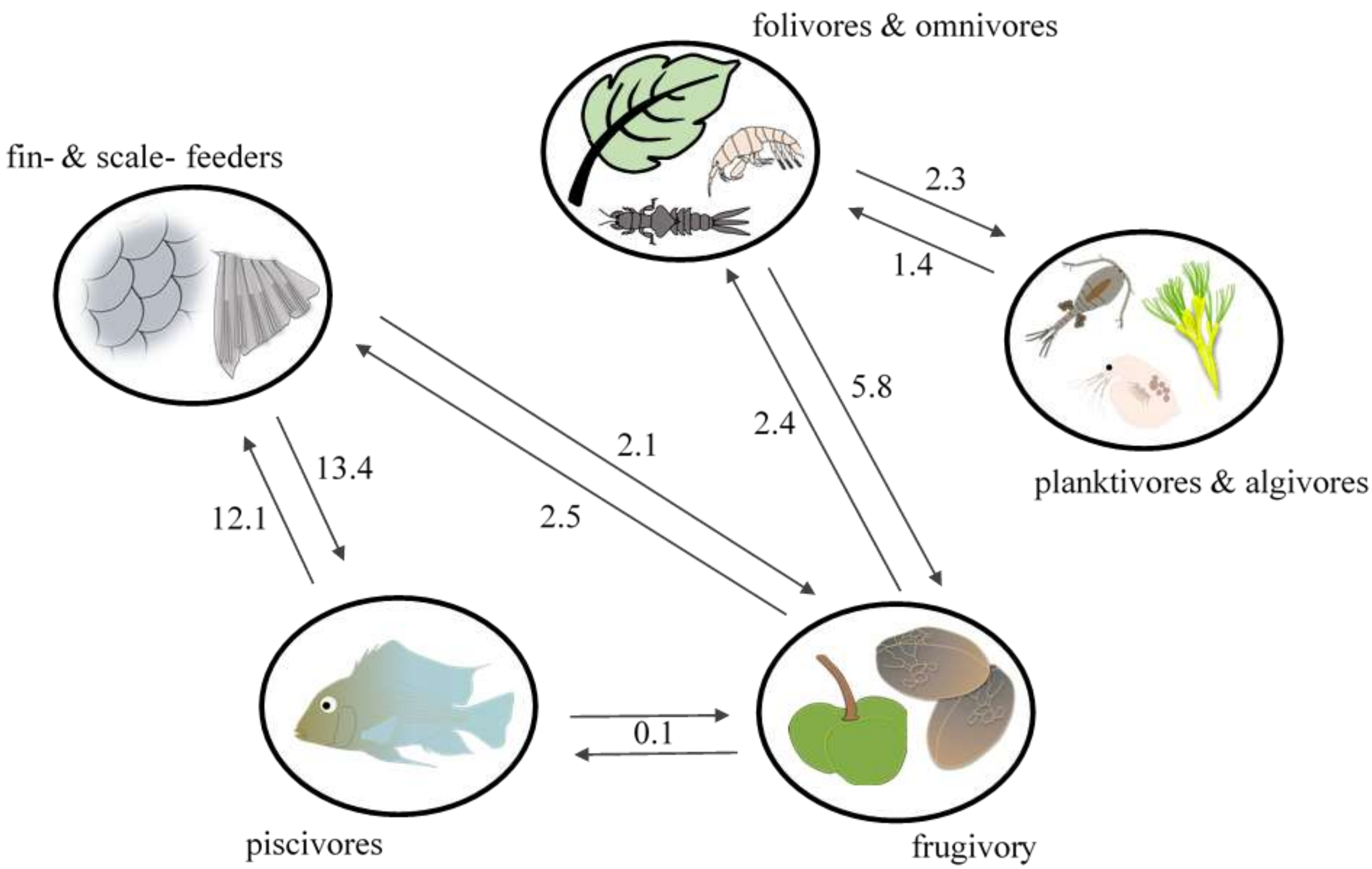
S5 - SUPPLEMENTAL TABLE & FIGURE– results for stochastic character mapping of diet states

frequency of changes between states (model = 'SYM')

x-y		fins.scales,fruits.seeds 2.1	fins.scales,folivore 0.0	fins.scales,piscivore 13.4	fins.scales,planktivore 0.0
x-y	fruits.seeds,fins.scales 2.5		fruits.seeds,folivore 2.4	fruits.seeds,piscivore 0.1	fruits.seeds,planktivore 0.0
x-y	folivore,fins.scales 0.0	folivore,fruits.seeds 5.8		folivore,piscivore 0.0	folivore,planktivore 2.3
x-y	piscivore,fins.scales 12.1	piscivore,fruits.seeds 0.1	piscivore,folivore 0.0		piscivore,planktivore 0.0
x-y	planktivore,fins.scales 0.0	planktivore,fruits.seeds 0.0	planktivore,folivore 1.4	planktivore,piscivore 0.0	

mean total time spent in each state is:

	fins.scales	fruits.seeds	folivore|omnivore	piscivore	planktivore|algivore
raw	33.4	94.8	215.2	43.2	31.3
%	8.0	22.7	51.5	10.3	7.5



(fruits.seeds = frugivory; folivore = folivores & omnivores). numerals represent transition frequencies.

S6 - DIET REFERENCES

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S7 - TAXONOMIC RECOMMENDATIONS & MORPHOLOGICAL SYNAPOMORPHIES

The current study provides robust molecular support for recognizing three major lineages of Serrasalmidae at the subfamilial rank: Colossomatinae (pacus common to lowland, white water habitats), Myleinae (pacus common to upland clear- and black water habitats), and Serrasalminae (*Metynnis* and piranhas, cosmopolitan). Likewise, recent molecular studies have helped place those taxa in a phylogenetic framework (Freeman et al. 2007; Ortí et al. 2008; Thompson et al. 2014) and uncovered new species-level diversity (Machado et al., 2018). Furthermore, there is strong support for the sister group relationship between Myleinae and Serrasalminae. Those results are consistent with previous phylogenies based on morphological (Cione et al., 2009) and molecular (Ortí et al., 2008; Thompson et al., 2014) data.

Among serrasalmins, the genus *Pristobrycon* Eigenmann 1915 has long been problematic for piranha taxonomy. The genus is considered an artificial (non-monophyletic) assemblage of at least six species divided into two groups, those with a preanal spine (*P. calmoni*, *P. aureus* and *P. eigenmanni*) and those without (*P. careospinus*, *P. maculipinnis* and *P. striolatus*) (Nico et al., 2018). The current study did not include *P. maculipinnis*, but placed the remaining species into three separate groups: '*aureus*' clade (*P. aureus*, *P. careospinus* and *P. eigenmanni*), sister group to monotypic genera *Catoprion* + *Pygopristis* (*P. striolatus*), and *Pristobrycon calmoni*, type species of the genus. Placement of *P. calmoni* varied between the three analyses. The concatenated amino acid analysis supported a sister group relationship between *P. calmoni* and the '*maculatus*' clade, whereas the concatenated nucleotide analysis placed it sister to the '*maculatus*' + '*rhombeus*' clade. The MSC analysis placed P. *calmoni* sister to the '*rhombeus*' clade. The simplest way to resolve the status of *Pristobrycon* is to expand the genus *Serrasalmus* to include *P. calmoni* as well as *P. aureus*, *P. careospinus*, *P. eigenmanni*. That said, *Pristobrycon striolatus* and the cryptic *P. scapularis* (Andrade et al., 2019) are not closely related to *Serrasalmus* and warrant a new generic name; until that time, we suggest the name '*Serrasalmus' striolatus* and 'S. *scapularis*' for the time being.

Among myleines, the genus *Tometes* is newly problematic. The genus includes seven species distributed in rivers draining the Guiana Shield into the Orinoco and Negro ('*T'. makue*), Amazon ('*T.' camunani* and *T. trilobatus* in part), and coastal rivers from the Maroni to the Araguari (*T. lebaili* and *T. trilobatus* in part) as well as rivers draining the Brazilian Shield into the Amazon ('*T.' ancylorhynchus*, '*T.' kranponhah* and '*T.' siderocarajensis*) (Andrade et al. 2017). In our analysis, two species from rivers draining the Brazilian Shield, *T. ancylorhynchus* and *T. kranponha*, are more closely related to species of *Mylesinus* and *Myleus* than to *Tometes* from coastal rivers draining the Guiana Shield, *T. lebaili* and *T. trilobatus* (type species). An analysis of an extensive dataset of DNA barcodes also failed support the monophyly of *Tometes* (Machado et al. 2018). The polyphyly of *Tometes* warrants further testing.

Finally, our analyses fail to support the exclusive monophyly of *Myloplus*, a result consistent with other molecular studies (Ortí et al. 2008; Thompson et al. 2014; Machado et al. 2018). Species currently assigned to *Myloplus* form up to six different lineages within Myleinae. For examples, '*Myloplus' schomburgkii* is the sister taxon to the monotypic *Ossubtus*, and '*M.'* cf. *lucienae* is the sister taxon to Brazilian Shield '*Tometes*.' '*Myloplus' arnoldi* is the sister taxon to a clade composed of '*M.' lucienae*, *Myleus (*ex. *Myloplus) planquettei*, Brazilian Shield '*Tometes'*, and species of *Mylesinus* and *Myleus*. *Myleus (*ex. *Myloplus) planquettei* nests within *Myleus* and members of this clade share number of morphological characteristics, notably an elongate cranium and wide-cusped teeth. *Prosomyleus (*ex. *Myloplus) rhomboidalis* is the sister taxon to all other myleins except *Acnodon*. *Myloplus rhomboidalis* (Cuvier 1818) is the type species of nominal subgenus *Prosomyleus* Géry 1972. Therefore, we recommend resurrecting *Prosomyleus* from the synonymy of *Myloplus* and elevating it to generic rank for species *Pr. rhomboidalis*. A similar scenario for *Paramyloplus ternetzi* (formerly *Myloplus*) and by association, its sister taxon *Paramyloplus* (formerly *Myloplus*) *taphorni* is outlined in the main text. All of our analyses also nested *Utiaritichthys* within this clade of true *Myloplus*. An analysis of DNA barcodes similarly nested *Utiaritichthys* well within *Myloplus* (Machado et al., 2018). *Utiaritichthys* is distinguished in part by its elongate bauplan, a feature it shares with *Myloplus asterias*.

Family Serrasalmidae: Günther, 1864

Morphological synapomorphies. The monophyly of this family is based on morphological synapomorphies proposed by Machado-Allison (1983, 1985) and by Buckup (1998). More recently, Kolmann et al. (2018) proposed the presence of a serrate, mid-ventral keel as being a synapomorphy for Serrasalmidae and subsequently, Kolmann et al. (2019) also proposed that unilateral tooth replacement is a synapomorphy for serrasalmids.

Comment. The subfamilies recognized in Serrasalmidae, Serrasalminae and Myleinae, follow Buckup (1998b) and Machado-Allison (1982, 1983, 1985). Myleinae and Serrasalminae were first proposed by Eigenmann (1915), with the former diagnosed by having two rows of premaxillary teeth and the latter having a single row of premaxillary teeth. Machado-Allison (1983) later modified these definitions, by proposing that the Myleinae be further distinguished by often having one pair of symphyseal teeth on the dentary and the Serrasalminae having tricuspid teeth. The major difference between Eigenmann's definition of the subfamilies *vs*. Machado-Allison's is that the former included *Catoprion* and *Metynnis* within the Serrasalminae.

Classification of Serrasalmidae

A new classification of suprageneric groups within Serrasalmidae is proposed based on the current molecular analysis (Fig. 2).

Family Serrasalmidae Bleeker 1859

- **Subfamily** Colossomatinae new subfamily*
  - **Included valid nominal genera:** *Colossoma* Eigenmann & Kennedy 1903, *Mylossoma* Eigenmann & Kennedy 1903, and *Piaractus* Eigenmann 1903
- **Subfamily** Myleinae Eigenmann 1903
  - **Included valid nominal genera:** *Acnodon* Eigenmann 1903, *Mylesinus* Valenciennes 1850, *Myleus* Müller & Troschel 1844, *Myloplus* Gill 1896 (includes *Utiaritichthys* Miranda Ribeiro 1937), *Ossubtus* Jégu 1992, and *Tometes* Valenciennes 1850
- **Subfamily** Serrasalminae Bleeker 1859
  - **Included valid nominal genera:** *Catoprion* Müller & Troschel 1844, *Metynnis* Cope 1878, *Prosomyleus* Géry 1972, Pygocentrus, *Pygopristis* Müller & Troschel 1844, and *Serrasalmus* Lacepède 1803 (includes *Pristobrycon* Eigenmann 1915).

*Putative synapomorphies uniting *Mylossoma*, *Colossoma*, & *Piaractus*:

- Generally with > 40 abdominal serrae (Machado-Allison, 1983; Kolmann et al., 2018)
- The dorsal fin not preceded by a spinous process continuous with the first pterygiophore (Machado-Allison, 1983)
- The intercalar bone is large and firmly attached to the neurocranium (Machado-Allison, 1983)
- Absence of a humeral hiatus in the anterolateral muscular body wall (Machado-Allison, 1983)
- Well-developed and elongate pterotic spine (Machado-Allison, 1983)
- Robust frontal bones with well-developed lateral extensions, which form a deep dilatator fossa (Machado-Allison, 1983)
- Robust, laterally-expanded mesethmoid (Machado-Allison, 1983)