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Structure and Function of the Armored Keel in Piranhas, Pacus, and Their Allies

MATTHEW A. KOLMANN,[1]* PAULINA URBAN,[2] AND ADAM P. SUMMERS[1]
[1]Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington, 98250
[2]Department of Biology, Christian-Albrechts University, Kiel, 24118, Germany

ABSTRACT

The serrasalmids: piranhas, pacus, and their relatives, are ubiquitous Neotropical fishes with diverse diets, ecologies, and behaviors. Serrasalmids have a bony, serrated keel which lines the underbellies of these fishes, the structure for which the family is named. We examined the diversity and structure of the keel in piranhas and allies using micro-computed tomography scanning in over 30 species of serrasalmids, a third of the species richness for the family, and for 95 total characiform specimens. The keel is highly diverse across serrasalmids, with serrae shape dictating the overall form of the keel. Serrae shape varies considerably among different species and even within keels themselves. The keel morphology can be divided into distinct anterior and posterior regions, as separated by the pelvic fins. Compared to other characiform fishes, serrasalmid skeletons are frequently damaged. Gouging perforations and signs of healing (serrae fusion) are common on the keel. We propose the keel is a defensive structure based on the high incidence of injury (>50%) in our dataset. This is the highest incidence of damage ever recorded in the skeletons of bony fishes. The loss of the anterior keel region in rheophilic taxa suggests competing performance demands and selective pressures on this structure. Competition and aggression among conspecifics or confamilials is a frequently invoked phenomenon for explaining animal weaponry and armor in terrestrial vertebrates. The keel in serrasalmids and other instances of armor in fishes could be complementary study systems for examining competitive rivalry in vertebrates. Anat Rec, 2018. © 2018 Wiley Periodicals, Inc.

Key words: characiformes; scutes; aggression; contest; pirapatinga; injury

INTRODUCTION

Starting with jawless fishes like heterostracans and osteostracans, fish armor function has been primarily attributed to defense against predation (Romer, 1933; Recher and Recher, 1968; Bell, 2001; Bell et al., 2004; Marchinko, 2009; Johanson et al., 2013; Price et al., 2015; but see Friedman and Sallan, 2012), whereas other explanations for armor, that is, an adaptation for combating negative buoyancy (Botella and Fariña, 2008; Fletcher et al., 2014), and/or use as an ion-sink, that is, as storage for mineral salts (Halstead, 1973; Ruben and Bennett, 1987) are invoked infrequently. Most of the earliest branching fish lineages were heavily armored with plated

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*Correspondence to: Friday Harbor Laboratories, niversity of Washington, Friday Harbor, Washington, 98250. Tel.: 360-320-8194. E-mail: kolmann@uw.edu

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bone (e.g., arthodire placoderms), while many of the earliest branching actinopterygians (amiiforms and polypteriforms; Allis, 1897, 1922; Bruet et al., 2008) have dense, scaled armor, and even comparably less-armored sturgeons have large bony scutes lining their bodies (Findeis, 1997). In these fishes, armor has been cited as a means of protection from predation (particularly from material scientists, e.g., Song et al., 2011; Yang et al., 2013, 2015), with little observational or experimental evidence (but see Reimchen, 1988, 1992, 2000). More recently, armored ostariophysans (catfishes) and percomorph fishes, have evolved robust armor in shallow-water lineages like syngnathiforms (e.g., syngnathids, pegasids; Porter et al., 2013), tetraodontiforms (pufferfishes and boxfishes; Brainerd, 1994), and even in deeper water lineages like monocentrids and aracanids. Despite extensive literature regarding other animal groups on the varied reasons for armor and animal weaponry, broad investigation into the diversity and function of armor across fishes have been limited (Stankowich, 2012).

More recent investigations of actinopterygian armor have uncovered surprising functional connotations; for example, unstable hydrodynamic armor in boxfishes makes for greater maneuverability at low speeds (Van Wassenbergh et al., 2015), while armor in *Polypterus* serves a respiratory function as an elastic energy storage device (Brainerd et al., 1989). However, much of the literature still assumes that armor protects fishes from their predators, particularly in materials and biomimetics research (Song et al., 2011; Yang et al., 2013). This idea has natural appeal, but stymies a more holistic understanding of morphological evolution; given the examples above, armor can clearly be selected for in other capacities, or at the very least, has multiple competing pressures which shape its evolution. If armor was suitable only for a single context, defense against predation, why such diversity in armor morphology among fishes?

The role of defensive weaponry and armor in aggressive interactions beyond predation is well-documented in other vertebrates (Stankowich, 2012), and we propose that competition with conspecifics and confamilials may lead to the evolution of armor in fishes. We are aware of only two studies which explicitly relate armor to interspecific aggression in fishes (both in sticklebacks; Kynard, 1979; Huntingford, 1981), but beyond these studies there has been little exploration of armor function as a defense against competitors. Based on the accounts of Teddy Roosevelt, piranhas and pacus (Serrasalmidae) gained notoriety for their aggressive "feeding frenzies," where low waters and scarce food resources would purportedly drive these predators to turn on one another when injured. These reports have now been dismissed as anecdotal and hyperbolic, while human injuries by piranhas are thought to stem from fish defending their nests during breeding season (Haddad and Sazima, 2003). However, piranhas do regularly "mutilate" other fishes as a feeding strategy, removing fins and chunks of flesh; and several piranha species engaging in mucus, fin, and scale-feeding (Sazima, 1988; Sazima and Pombal Jr, 1988; da Silva et al., 2015). Piranhas themselves are not strangers to similar kinds of aggression, being territorial fishes with robust dentition (Sazima, 1988; Fig. 1), and even in the manner Roosevelt described, will attack one another during pitched foraging and territorial disputes (Sazima and Machado, 1990). Given the strength of serrasalmid jaws, these sorts of intraspecific "discussions" between serrasalmid individuals have the potential for deadly or grievously-injured outcomes (Haddad and Sazima, 2003; Grubich et al., 2012).

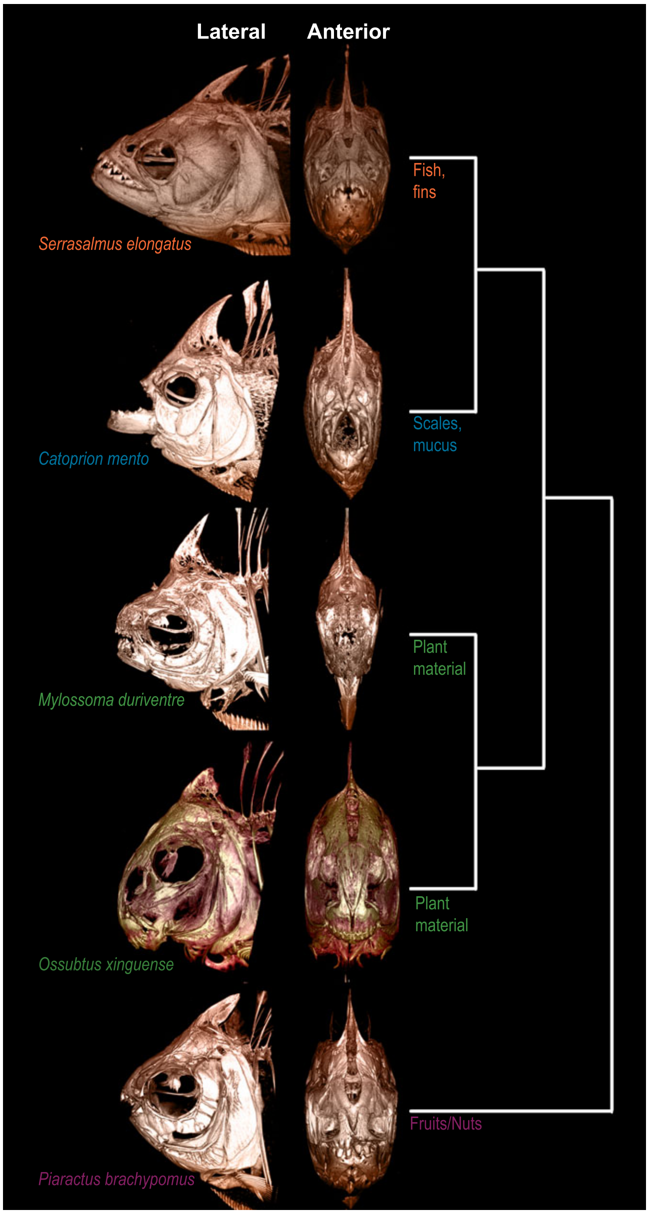

Fig. 1. Lateral detail of the cranium in five species of serrasalmids.

In serrasalmids, as with other characiform fishes, interspecific (confamilial) and intraspecific fin-nipping and other aggressive interactions are common (Sazima, 1983, 1988; da Silva et al., 2015). Aggression among fishes can generally be broken into three categories: predation, competition, and parasitism. Competition is delimited as either interspecific or intraspecific, and in serrasalmids there are documented cases of both intrafamilial (i.e., 'interspecific') and intraspecific aggression, in addition to scale-feeding parasitism by some piranhas on other serrasalmids (Northcote et al., 1986; Sazima and Machado, 1990). Sazima and Machado (1990), looking at four species of serrasalmids in the wild, found confrontation to be routine, if typically not deadly. Frequent mutilation of other fishes by piranhas, particularly cichlids and erythrinids, has purportedly prompted the evolution of caudal ocelli in potential prey like *Cichla* (Winemiller, 1990). This is supposed to deter piranhas from their typical attack behavior, approaching prey from underneath and behind to crop fin rays from tails (Northcote et al., 1986; Sazima and Pombal Jr, 1988). Given this tendency of piranhas to attack the underside and tails of prey fishes, perhaps it is no surprise that they have a robust bony keel lining their underbelly.

The genus *Serrasalmus* and the family Serrasalmidae more broadly, were named by Lacépède (1803) for the exaggerated serrations or "serrae" of this keeled structure (Fig. 2). The keel has been used by systematists to distinguish between certain species of serrasalmids, most notably herbivorous genera like *Tometes, Myloplus, Myleus,* and *Utiarichthys* (Pereira and Castro, 2014; Andrade et al., 2016 a,b, Andrade et al., 2017). The keel is highly variable: robust, stretching from anus to gular region in some lineages; while a curiously truncated, post-pelvic remnant exists in some rheophilic taxa like *Tometes* and *Ossubtus.* The keel superficially resembles the dermal armor in well-armored fishes such as poachers, sea moths, pipefishes, and seahorses (Pietsch, 1978; Britz and Johnson, 2002).

The aggressive nature of piranhas and pacus, coupled with their territoriality over feeding grounds and nesting sites, make these fishes an interesting system for examining whether fish armor can have another role: as defense against confamilials. Despite the keel having been described over some 200 years ago, its function is entirely unknown, nor has its morphology been described in-depth across serrasalmids. The aims of this study are four-fold (1) demonstrate the variation in keel morphology across serrasalmids, (2) generate a detailed description of the keel and individual serrae as they vary along the ventrum, (3) quantify the distribution of keel morphology in the context of phylogeny, and (4) highlight examples of skeletal damage attributable to partial predation and/or aggressive interactions. Our prediction is that damage to the keel is more frequent than elsewhere in the skeleton, consistent with a defensive role to these structures.

MATERIALS AND METHODS

We used micro-source computed tomography scanning (µCT; Bruker Skyscan 1,173, Billerica, MA) at the Karel Liem Bioimaging Facility (Friday Harbor Labs, WA) to

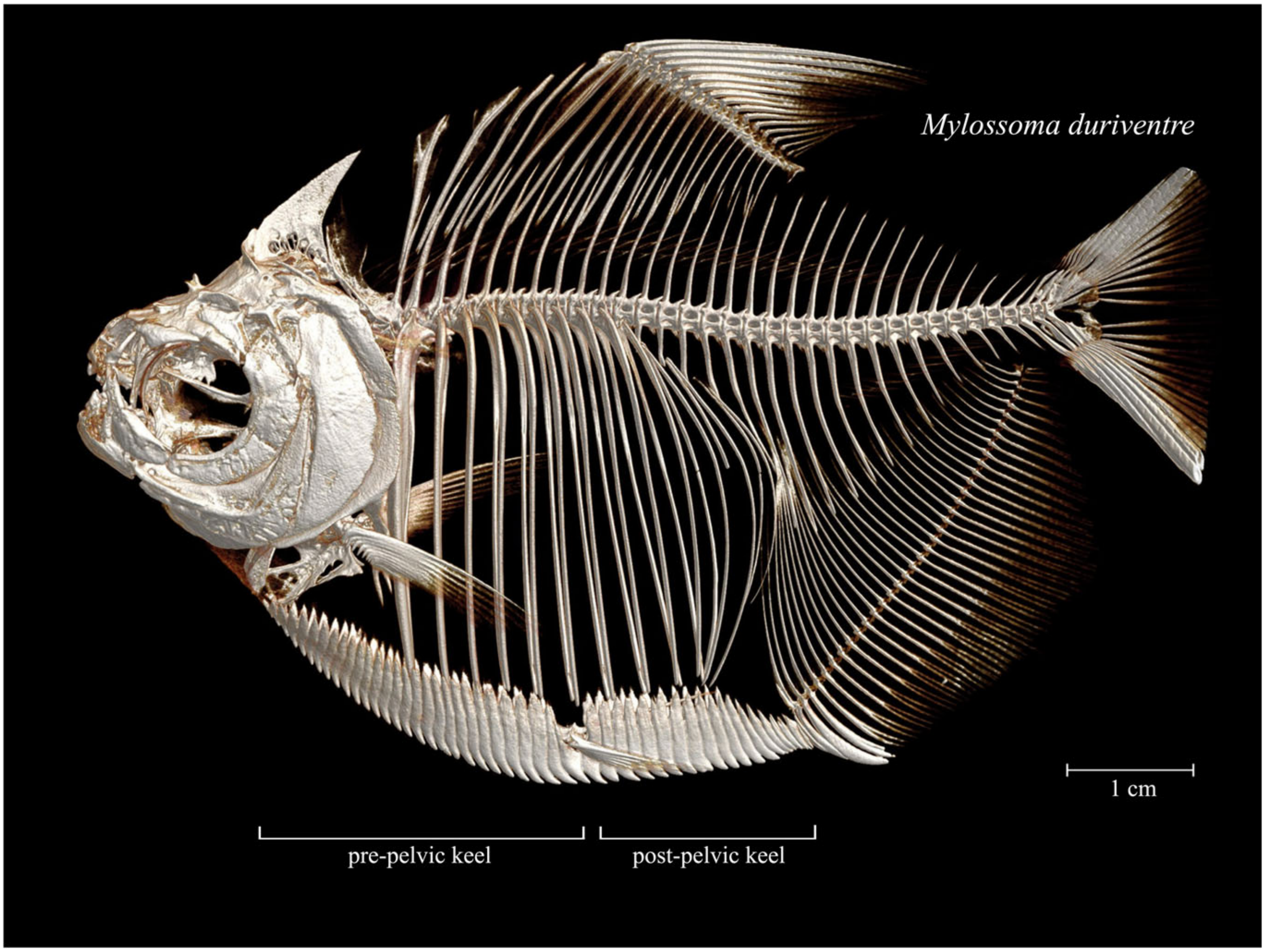

Fig. 2. Whole skeleton of *Mylossoma duriventre*, detailing the keel and serrae.

TABLE 1. Serrasalmids and other characiform genera surveyed in this study

Serrasalmidae	Other characiforms
Colossoma	*Agoniates*
Mylossoma	*Astyanax*
Piaractus	*Chalceus*
Acnodon	*Charax*
Mylesinus	*Cynodon*
Myleus	*Cyphocharax*
Myloplus	*Hyphessobrycon*
Tometes	*Hypomasticus*
Ossubtus	*Leporinus*
Metynnis	*Moenkhausia*
Catoprion	*Prochilodus*
Pristobrycon	*Pseudanos*
Pygopristis	*Rhaphiodon*
Serrasalmus	*Roeboides*
Pygocentrus	*Synaptolaemus*
	Thorachocharax
	Triportheus

examine keel morphology in 70 museum specimens representing 37 species or 38.9% of serrasalmid species diversity (Table 1). We also scanned 25 specimens of other characiform fishes for comparative purposes (Table 1). Scans used a 1 mm aluminum filter and ranged in voxel size from 17.1 to 62.0 um, with beam settings ranging from 60–70 kV and 100–133 uA, on a 2048 × 2048-pixel CCD. Specimens were wrapped with cheesecloth moistened with ethanol (70%), inside 3D-printed PVA plastic tubes to prevent movement during scanning. Resulting μCT data were visualized, measured, segmented, and rendered using Horos (www.horosproject.org) and Amira (FEI) softwares.

Several morphometric measures were taken to characterize the size, shape, and regional variation of the ventral keel. We measured whole keel length, pre-pelvic and post-pelvic keel length, average serrae height and width. We also calculated the aspect ratios for serrae from pre-pelvic and post-pelvic keel regions independently (Fig. 2). We recorded the total number of serrae per keel, and the number of serrae per keel region. Finally, we used the published serrasalmid phylogeny from Thompson et al. (2014) to examine trends in keel aspect ratio across the family and whether keels from closely-related species were more similar to one another than expected by chance alone. Tests of phylogenetic signal (significance: $P \leq 0.05$) were executed using the (phylosig) command in the *geiger* package (Harmon et al., 2007). We visually inspected both serrasalmids (n = 68) and other characiform fishes (n = 25) using volume renders in CTVox (Bruker, Billerica, MA) and Amira for injury, damage, or skeletal aberrations to the head, keel, and rest of the skeleton (Table 2).

RESULTS

General Principles of Keel Morphology

The serrasalmid keel is a bony structure extending from the gular region to the anus and in most cases, bridges the pelvic fin girdle. The keel is partially covered by scales and integument with only the most distal cusps of the serrae externally visible in most serrasalmid species. In general, the overall shape of the keel is either ovoid or strongly pointed, with more ovoid shapes characterizing thicker (less laterally-compressed) taxa (*Pygocentrus caribe, P. nattereri, Pristobrycon*) and pointed keels in higher-bodied, narrower species (e.g., *Myleus setiger, Catoprion mento*) (Figs. 3–5). Individual serrae form the imbricated segments of the keel, with serrae slightly overlapping at their base and the tips. Serrae are distally pointed and flare to an inverted chevron, either a "Y" or 'V' shape, dorso-laterally (Figs. 3–5). This dorsolateral flaring becomes more exaggerated posteriorly as the trunk thickens, and then becomes reduced posterior to the pelvic fins as the body tapers to the caudal peduncle (Figs. 3–4).

The degree to which individual serrae overlap and become imbricated varies considerably among serrasalmids. These articulations range from total overlap in species like *Mylossoma duriventre* and *Pygocentrus nattereri*, to the condition in *Pristobrycon calmoni*, where anterior serrae have noticeable gaps in between. The lateral margins of these serrae vary considerably in how smooth and orderly imbrication proceeds, with serrae that are largely similar in shape and size as in *M. duriventre* (Fig. 3A), to more haphazardly joined keels as in *Pristobrycon calmoni* and *P. striolatus* (Fig. 3C). Serrae cusps also vary within and among species in the degree to which the distal 'cusp' is recurved and whether this recurve lies flush with the cusps of adjacent serrae. The serrae cusps in fishes like *Pristobrycon* form strongly recurved, even hooked distal tips while *Pygocentrus* and *Piaractus* have such smoothly interlocking serrae that the distal cusps also imbricate (Fig. 3A,C lateral).

The shape of individual serrae, as well as the keel in general, can vary drastically from anterior to posterior (Figs. 3–5). In general, the serrae posterior to the pelvic fins become taller in aspect ratio commensurate with overall body narrowing while anterior (pre-pelvic fin) serrae have broader lateral flanges (Fig. 3A–D, dorsal and ventral). Some serrasalmids even lack keel armor anterior to the pelvic fins, particularly in species with comparably large pelvic fins (e.g., *Ossubtus xinguense, Acnodon normani*, Fig. 3B). In *O. xinguense, Tometes kranponhah,* and *Catoprion mento*, the post-pelvic keel as a whole changes drastically (Figs. 3B,C, 4–5), with the posterior-most serrae in these species bifurcating to flank rather than envelop the ventral surface of the fish. In these bifurcating serrae, one of the lateral flanges is lost and the cusps of the now two individual serrae are aligned along the midline. However, the post-pelvic serrae are narrower than anterior serrae, and imbricate more tightly, even in those species for which the anterior serrae are not strongly interlocking (e.g., in *Pristobrycon*).

Systematic Considerations of Keel Morphology

We use the Thompson et al. (2014) tree to organize serrasalmids into six clades: (1) a clade including *Mylossoma, Colossoma,* and *Piaractus*; (2) a clade including *Myloplus, Tometes, Mylesinus, Ossubtus,* and *Acnodon*; (3) a clade including *Metynnis* species; (4) a clade including *Catoprion mento, Pygopristis denticulata,* and *Pristobrycon striolatus*; (5) a clade including *Pygocentrus* species; and (6) a clade including *Serrasalmus* species and *Pristobrycon calmoni.*

Clade 1: These pacus, tambaqui, and pirapatingas generally have many tall, thin serrae numbering in our dataset from 41–56. Overall the keel in these taxa is pointed on either end, although the anterior keel of *Mylossoma duriventre* flares laterally, giving it a more

TABLE 2. Instances of damage to serrasalmid and other characiform fishes surveyed in this study

			Damage: Keel & Serrae			Damage: Non-Keel		
Species	Museum	Catalog #	Flange Loss	Fusion	Perforations	Jaw	Opercle	Ribs
Colossoma macropomum	SU-CAS	55,894	NA	NA	NA	NA	N	N
Colossoma macropomum	SU-CAS	55,894	NA	NA	NA	NA	N	N
Colossoma macropomum	FMNH	78,087	Y	Y	N	Y	Y	N
Colossoma macropomum	FMNH	78,087	N	N	N	N	N	N
Colossoma macropomum	FMNH	78,087	Y	Y	N	Y	N	N
Colossoma nigripinnis	CAS	69,233	N	Y	N	N	N	N
Mylossoma duriventre	CAS	15,675	N	N	N	Y	N	N
Mylossoma duriventre	CAS	15,675	N	N	N	Y	N	N
Mylossoma duriventre	CAS	15,675	N	N	N	Y	N	N
Mylossoma duriventre	CAS	15,675	N	N	N	Y	N	N
Mylossoma duriventre	SU-CAS	54,683	N	N	N	N	N	N
Mylossoma duriventre	SU-CAS	54,683	N	Y	N	N	N	N
Mylossoma duriventre	CAS	54,683	N	Y	N	N	N	Y
Piaractus brachypomus	ANSP	166,685	Y	Y	N	Y	N	N
Acnodon normani	CAS	20,739	N	N	N	N	N	N
Mylesinus paucisquamatus	CAS	20,221	Y	Y	N	N	N	N
Myleus setiger	ANSP	197,912	Y	Y	N	N	N	N
Myloplus schomburgkii	SU-CAS	70,039	Y	Y	N	N	N	N
Myloplus rhomboidalis	SU-CAS	34,504	N	N	N	N	N	N
Myloplus rubripinnis	ANSP	199,578	NA	NA	NA	N	N	N
Myloplus rubripinnis	TCWC	8,411.02	NA	NA	NA	NA	N	N
Myloplus levis	FMNH	56,928	Y	Y	N	Y	N	N
Myloplus asterias	FMNH	69,916	Y	Y	N	N	N	N
Myloplus asterias	FMNH	69,916	Y	Y	N	N	N	N
Myloplus torquatus	FMNH	109,794	Y	Y	N	N	N	N
Metynnis altidorsalis	FMNH	97,720	NA	NA	NA	NA	N	N
Metynnis luna	CAS	11,770	N	Y?	N	N	N	N
Metynnis hypsauchen	SU-CAS	59,384	N	N	N	N	N	N
Metynnis hypsauchen	FMNH	56,916	N	N	N	N	N	N
Tometes kranponhah	ANSP	196,745	N	N	N	N	N	N
Tometes kranponhah	ANSP	196,745	Y	Y	N	N	N	N
Ossubtus xinguense	ANSP	197,392	N	N	N	N	N	N
Catoprion mento	CAS	20,216	Y	Y	Y?	N	Y	N
Catoprion mento	CAS	20,216	Y	Y	N	N	N	N
Catoprion mento	ROM	95,239	Y	Y	N	N	N	N
Pristobrycon striolatus	ANSP	166,906	N	Y	N	Y	N	N
Pygopristis denticulata	CAS	20,208	N	Y	N	N	Y	N
Pristobrycon calmoni	CAS	15,277	Y	N	N	N	N	N
Pristobrycon calmoni	CAS	15,277	NA	NA	NA	NA	N	N
Serrasalmus eigenmanni	SU-CAS	21,982	N	Y	N	N	N	N
Serrasalmus manueli	ANSP	198,551	Y	Y	Y	N	N	N
Serrasalmus medinai	ANSP	165,387	Y	Y	Y	Y	N	Y
Serrasalmus medinai	TCWC	7,503.06	Y	Y	N	N	N	Y
Serrasalmus spilopleura	SU-CAS	16,773	NA	Y	NA	NA	N	N
Serrasalmus spilopleura	SU-CAS	16,773	NA	NA	NA	NA	N	N
Serrasalmus spilopleura	FMNH	108,506	N	N	N	N	N	N
Serrasalmus spilopleura	FMNH	108,506	Y	N	N	N	N	N
Serrasalmus irritans	FMNH	100,130	N	N	N	N	N	N
Serrasalmus irritans	FMNH	100,130	N	N	N	N	N	N
Serrasalmus brandti	FMNH	56,945	N	N	N	N	N	N
Serrasalmus brandti	FMNH	56,945	N	N	N	N	N	N
Serrasalmus maculatus	FMNH	56,976	N	N	N	N	N	N
Serrasalmus maculatus	FMNH	56,976	Y	N	Y	Y	Y	N
Serrasalmus rhombeus	FMNH	111,315	Y	Y	N	N	N	N
Serrasalmus rhombeus	FMNH	111,315	Y	Y	N	N	N	N
Serrasalmus marginatus	FMNH	71,030	N	N	N	N	N	N
Serrasalmus elongatus	FMNH	111,314	Y	Y	Y	N	N	N
Serrasalmus elongatus	FMNH	111,314	Y	Y	Y	N	Y	N
Serrasalmus humeralis	FMNH	56,969	Y	Y	N	N	N	N
Serrasalmus humeralis	FMNH	56,969	Y	N	Y	Y	N	N
Pygocentrus caribe	FMNH	103,670	N	Y	Y	N	Y	N
Pygocentrus caribe	FMNH	103,670	N	N	Y	N	N	N
Pygocentrus nattereri	FMNH	111,306	Y	N	N	Y	N	N
Pygocentrus nattereri	FMNH	111,306	N	N	N	Y	N	N
Pygocentrus nattereri	FMNH	111,306	N	Y	N	N	N	N

(Continues)

TABLE 2. Continued

			Damage: Keel & Serrae			Damage: Non-Keel		
Pygocentrus natterei	CAS	71,016	Y	Y	Y	Y	Y	N
Pygocentrus piraya	CAS	71,018	Y	Y	N	Y	N	N
Pygocentrus piraya	CAS	71,018	Y	Y	N	N	N	N
Other Characiforms (non-serrasalmids) Surveyed								
Cynodon gibbus	FMNH	104,995	N	N	N	N	N	N
Rhaphiodon vulpinis	FMNH	78,756	N	N	N	N	N	N
Cynodon gibbus	FMNH	104,995	N	N	N	N	N	N
Rhaphiodon vulpinis	FMNH	78,756	N	N	N	N	N	N
Triportheus sp.	CAS	50,776	N	N	N	N	N	N
Chalceus macrolepidotus	CAS	11,872	N	N	N	N	N	N
Triportheus elongatus	CAS	11,837	N	N	N	N	N	N
Leporinus nigrotaeniatus	OSU	18,498	N	N	N	N	N	N
Pseudanos trimaculatus	USNM	280,740	N	N	N	N	N	N
Leporinus taeniatus	UMMZ	216,434	N	N	N	N	N	N
Leporinus ortomaculatus	AUM	35,678	N	N	N	N	N	N
Charax cf. pauciradiatus	AUM	45,439	N	N	N	N	N	N
Charax cf. pauciradiatus	AUM	45,439	N	N	N	N	N	N
Charax cf. pauciradiatus	AUM	45,439	N	N	N	N	N	N
Roeboides affinis	AUM	44,837	N	N	N	N	N	N
Roeboides affinis	AUM	44,838	N	N	N	N	N	N
Synaptolaemus cingulatus	AUM	54,407	N	N	N	N	N	N
Hypomasticus megalepis	OSU	18,497	N	N	N	N	N	N
Cyphocharax spiluropsis	TCWC	13,995.04	N	N	N	N	N	N

rounded aspect in ventral perspective (Fig. 3A). The serrae cusps themselves are densely-overlapping in *Piaractus*, less densely-overlapping in *Colossoma* species, and strongly differentiated in *Mylossoma*, leading to a conspicuous saw blade-like keel in this latter taxon. In *Mylossoma, Colossoma,* and *Piaractus* the serrae are strongly imbricated, with no obvious gaps between articulated elements. Serrae counts are 42–49 in *Colossoma macropomum*, 47 in *C. nigripinnis*, 41–53 in *Mylossoma duriventre*, and 56 in *Piaractus.* There is little overall asymmetry between the anterior and posterior keel and serrae shape in this clade, with anterior serrae resembling posterior serrae, with anterior serrae generally smaller in size (Fig. 3A).

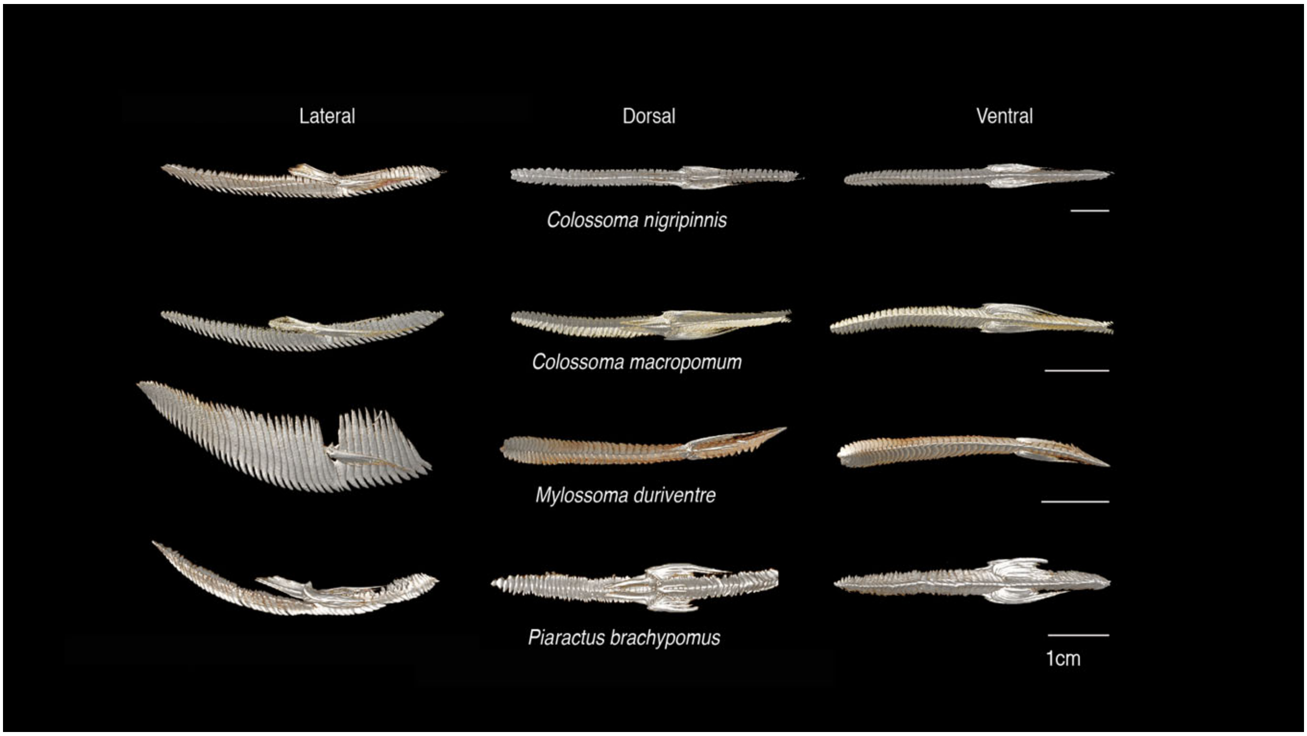

Fig. 3. Morphological variation in the structure of keel armor across Serrasalmidae. From left to right: lateral view; dorsal view; ventral view. Scale bars specific to each individual specimen. Anterior of the keel is to the left of the figure.

Fig. 3. Continued

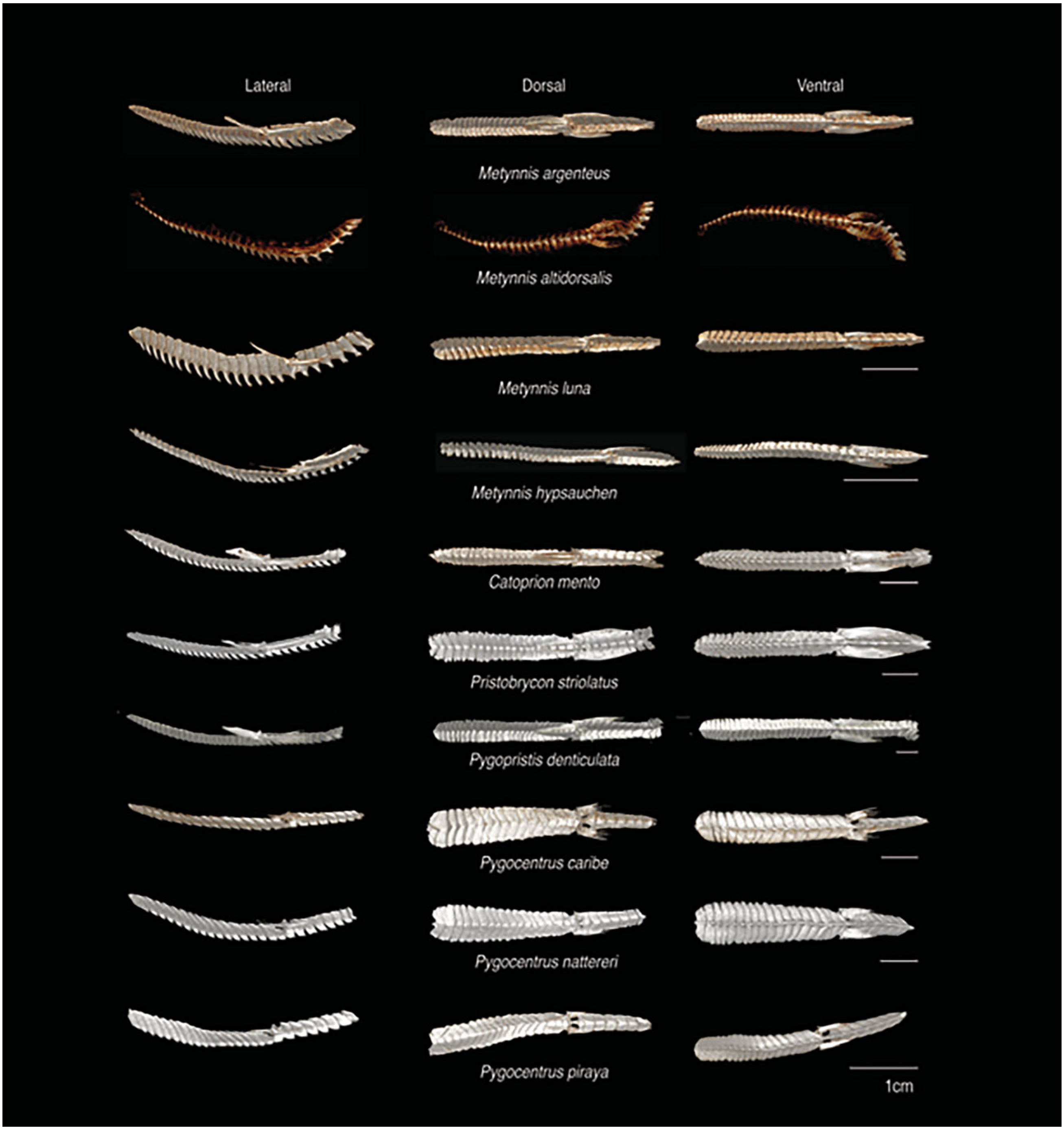

Fig. 3. Continued

Clade 2: These pacus (*Myloplus, Tometes, Mylesinus, Ossubtus,* and *Acnodon*) have overall keel shapes similar to the larger pacus, tambaqui, and pirapatinga in Clade 1; pointed at both ends and with similar serrae shapes in both pre- and post- pelvic regions (Fig. 3B). The most obvious difference among taxa is the total loss of the serrae anterior to the pelvic girdle in *Acnodon* and *Ossubtus*, as well as stark reductions in pre-pelvic serrae size, symmetry, and overall anterior keel length in *Mylesinus, Myloplus rubripinnis,* and *Tometes kranponhah.* Commensurate with either reduction or total loss of the anterior keel, the lateral stays of the pelvic girdle extend far anteriorly, moving outside the bounds of the keel, while in other serrasalmids the pelvic girdle is contained within the lateral confines of the keel armor (Fig. 3B). For those species with reduced pre-pelvic keels, the serrae are narrow and become progressively reduced in size anteriorly. Compared to Clade 1, we see greater differentiation

Fig. 3. Continued

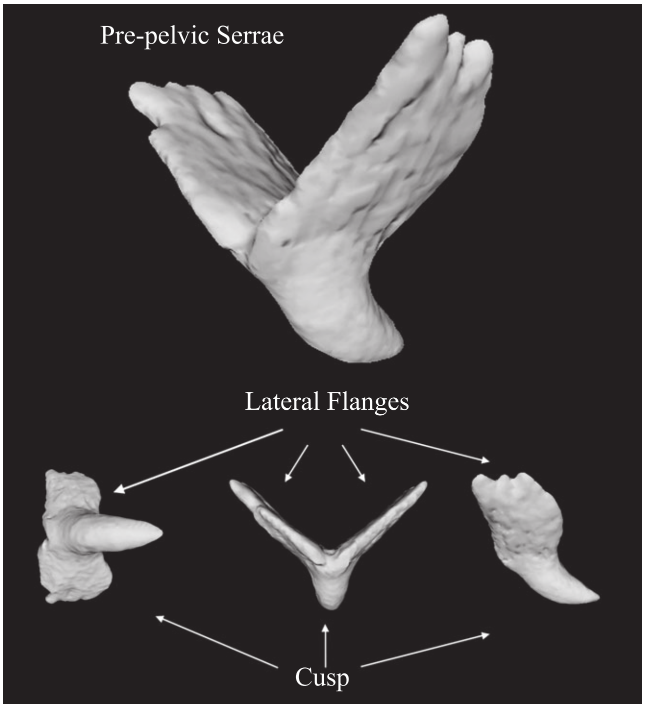

Fig. 4. Morphology of the anterior (pre-pelvic) serra from *Catoprion mento.* Top models are fully rotatable models. To activate the model, click on the pelvic serra in the center of the page. The model can then be freely manipulated, and structures can be rendered transparent by right clicking the object. To open Figure 4 in a floating window that will remain open throughout the document, after the 3D PDF is activated (click to activate), right click (or control-click) on the model, then click View in Floating Window. The 3D PDF will remain open in a resizable floating window.

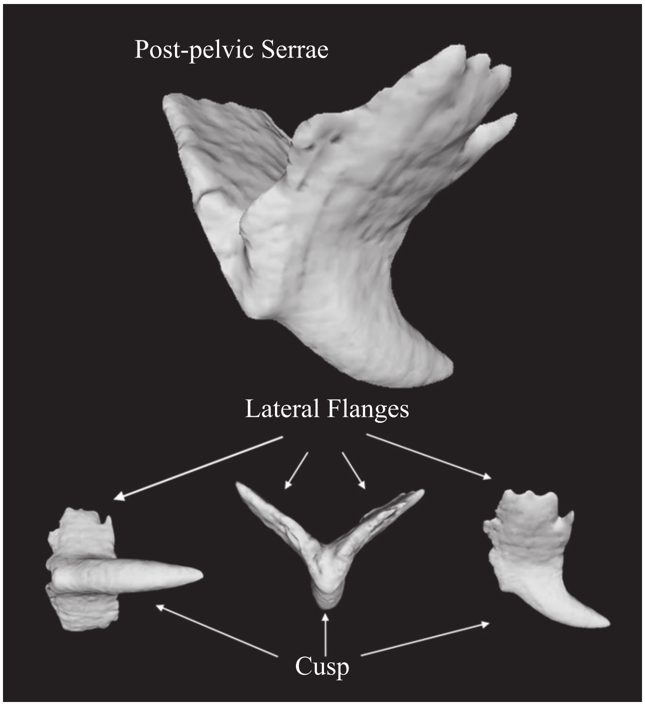

Fig. 5. Morphology of the posterior (post-pelvic) serra from *Catoprion mento.* Top models are fully rotatable models. To activate the model, click on the pelvic serra in the center of the page. The model can then be freely manipulated, and structures can be rendered transparent by right clicking the object. To open Figure 4 in a floating window that will remain open throughout the document, after the 3D PDF is activated (click to activate), right click (or control-click) on the model, then click View in Floating Window. The 3D PDF will remain open in a resizable floating window.

between pre-pelvic and post-pelvic serrae, with posterior serrae generally wider, taller, and more robust than anterior serrae in taxa like *Acnodon*, *Mylesinus, Myleus, Myloplus rubripinnis, Tometes,* and *Ossubtus.* This pattern correlates with the posterior-most serrae becoming bifurcated and flanking the anal fin in taxa like *Myleus setiger, Myloplus levis, M. asterias, M. torquatus, Tometes kranponhah,* and *Ossubtus* (Fig. 3B).

Serrae counts for the *Myloplus* + allies clade were: 49 in *Myloplus levis,* 48 serrae in *M. torquatus,* 37–46 in *M. asterias* (serious damage to the middle keel in one specimen), 40 in *M. rhomboidalis,* 32 in *M. schomburgkii*, 26 in *Myloplus rubripinnis,* 28 in *Mylesinus paucisquamatus*, 13 in *Ossubtus xinguense*, 44 in *Myleus setiger*, 29–37 in *Tometes kranponhah,* and eight in *Acnodon normani.* The serrae cusps themselves are densely-overlapping in *Myloplus levis, M. asterias*, *M. torquatus, Mylesinus*, and *Ossubtus*, less densely-overlapping in *Myleus*, *Tometes, Acnodon,* and *Myloplus rubripinnis*, and strongly differentiated in *Myloplus schomburgkii* and *M. rhomboidalis*, leading to a more piranha-like, serrated keel in these last two taxa (Fig. 3B). In general, the degree of serrae overlap is more pronounced anteriorly, while in post-pelvic serrae, cusps are more differentiated. In *M. paucisquamatus* and to a larger extent, *M. schomburgkii,* serrae are not well-imbricated, with obvious gaps between articulated elements, although this could be a consequence of poor mineralization on serrae flanges (Fig. 3B).

Clade 3: *Metynnis* species have well-overlapping serrae flanges with strongly protruding serrae cusps. Serrae aspect in cross-section is chevron-shaped (Fig. 3C). The gap between serrae cusps varies conspicuously between taxa, with the most widely-spaced cusps in *Metynnis luna and M. altidorsalis*, and more recurved and closely-packed cusps in *M. argenteus* and *M. hypsauchen.* Serrae number was 34 in *Metynnis argenteus,* 33 in *M. altidorsalis,* 25 in *M. luna,* and 30–34 in *M. hypsauchen.* Overall the keel in these taxa is blunted on either end, with only the last post-pelvic serrae showing any sign of bifurcating (Fig. 3C). In general, serrae overlap is similar between pre-pelvic and post-pelvic regions, with posterior serrae slightly taller than anterior serrae, and cusp shape is largely similar between pre-pelvic and post-pelvic regions. However, there is stark contrast between the pre-pelvic and post-pelvic serrae cusp morphology in *Metynnis hypsauchen*; anterior cusps are strongly overlapping while posterior cusps are noticeably separated by large gaps (Fig. 3C).

Clade 4: These piranhas, *Catoprion, Pygopristis,* and *Pristobrycon striolatus*, have serrae shaped like equilateral chevrons in cross-section, but serrae which are generally wider than serrae in Clades 1, 2, and 3, and with more recurved (falciform) serrae cusps. Serrae number was 30–36 (mean = 33) in *C. mento,* 37–44 (mean = 40) in *P. denticulata,* and 29 in *P. striolatus* (Fig. 3C). Overall the keel in these taxa is blunted on either end, with only the last two or three post-pelvic serrae bifurcating to extend around the anal fin insertion. In general, there is very little obvious differentiation in serrae shape between pre-pelvic and post-pelvic regions (Fig. 3C). The serrae cusps themselves are less densely-overlapping in *Catoprion* and *P. striolatus*, having a saw-like appearance in lateral perspective than in *P. denticulata*, where serrae cusps broadly overlap. Serrae flanges are wider in *P. striolatus* and *P. denticulata* than in *Catoprion*, reflecting the more laterally-compressed body plan in *Catoprion* over its sister taxa. In general, serrae overlap is similar between pre-pelvic and post-pelvic regions and cusp shape is largely similar between anterior and posterior serrae (Fig. 3C).

Clade 5: *Pygocentrus* species have the widest and flattest serrae, which are shaped more like hemispheres in ventral perspective, than chevron-shaped as in other piranhas. Serrae cusps are obvious but not strongly recurved (Fig. 3C). Serrae number was 24–26 in *P. caribe,* 24–26 (mean = 25) in *P. nattereri,* and 26 in *P. piraya.* The keel in these taxa is blunted on either end, with only the last post-pelvic serrae showing any sign of bifurcating. There is stark contrast between pre-pelvic and post-pelvic serrae shape, with anterior serrae broad and flattened (except the cusps) and posterior serrae more laterally-compressed and chevron-shaped in cross-section (more typical of other serrasalmids). In general, serrae overlap is similar between pre-pelvic and post-pelvic regions, although slightly more pronounced anteriorly, and cusp shape is largely similar between anterior and posterior serrae (Fig. 3C).

Clade 6: These piranhas (*Serrasalmus* and *Pristobrycon calmoni*) have overwhelmingly similar serrae and overall keel shapes between taxa relative to other serrasalmids. The keel in these taxa is blunted on either end, with only the last post-pelvic serrae bifurcating to extend around the anal fin insertion (Fig. 3D). Serrae cross-sectional shape is chevron-shaped, with narrower serrae flanges than *Pygocentrus*, but generally wider than the serrae in Clades 1–3. Serrae cusps are uniformly pronounced and slightly recurved, with taxa like *Serrasalmus marginatus, S. maculatus,* and *S. elongatus* having the least-pronounced cusps. Serrae number ranged between 27–35 (mean = 32) in *Serrasalmus* species and 30–31 in *Pristobrycon calmoni.* Serrae number was 34 in *Serrasalmus maculatus* and *S. elongatus*, 31 in *S. marginatus* and *S. eigenmanni,* 28–35 (mean = 31) in *S. medinai*, 27–35 (mean = 31) in *S. spilopleura*, 31–33 in *S. brandti*, 29–32 in *S. humeralis,* and 29–31 in *S. irritans.* In *Serrasalmus*, post-pelvic serrae overlap more fully than anterior serrae (Fig. 3D). However, *P. calmoni* has anterior serrae segments which do not articulate, while serrae adjacent to the pelvic girdle imbricating more wholly. The serrae cusps in these taxa have noticeable gaps in between, mirroring the weakly-imbricate nature of the serrae themselves. More pronounced serrae cusps predominate in *P. calmoni, S. eigenmanni, S. spilopleura, S. irritans,* and *S. rhombeus.* In general, anterior serrae are wider than posterior serrae, particularly in *S. maculatus*, a wide-bodied piranha, whose anterior serrae look strikingly similar to *Pygocentrus* serrae (Figs. 3C,D).

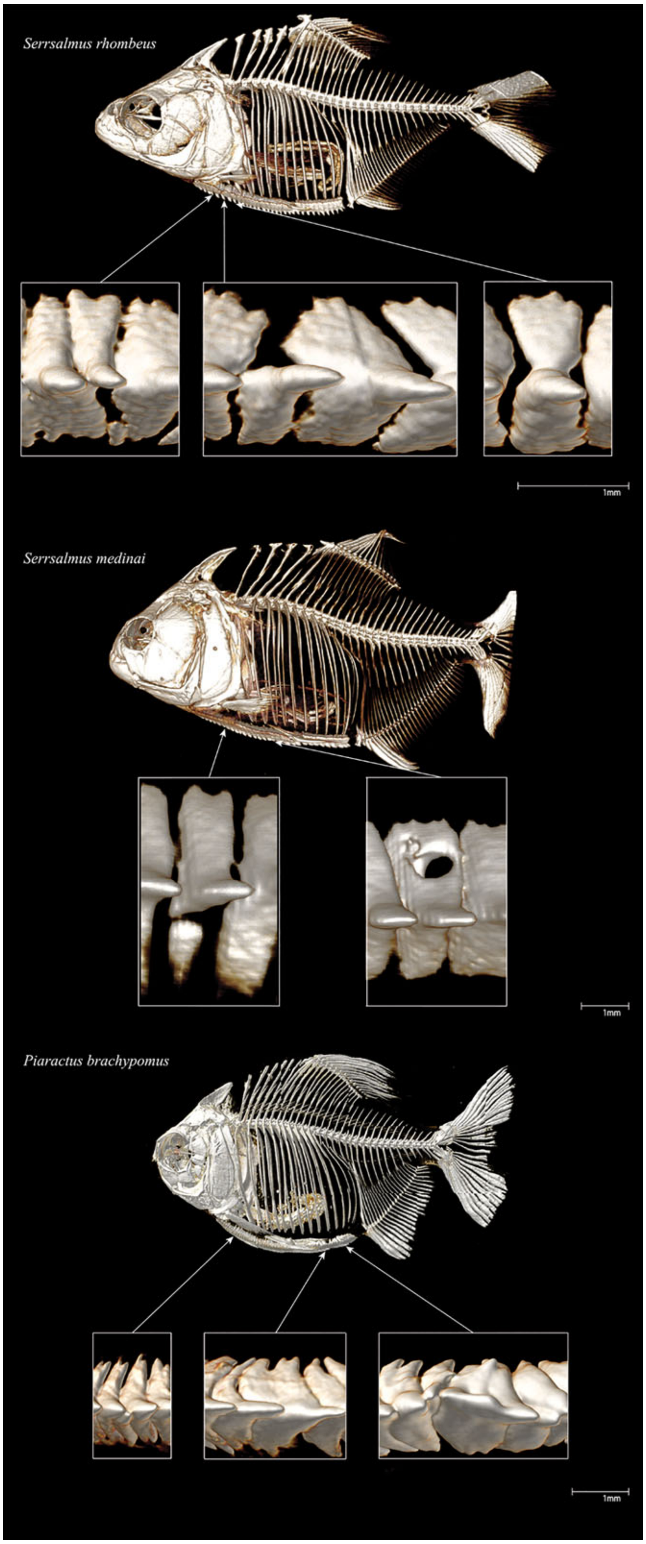

Fig. 6. Examples of injury to the keel in three species of serrasalmids.

Phylogenetic Patterns in Keel Morphology & the Influence of Rheophily

Serrae number was strongly predicted by phylogeny (K = 0.72, p = 0.03; lambda = 0.94, p = 0.001), but serrae aspect ratio and keel length showed no immediate correlations with phylogeny. Post-pelvic keel length showed weak association with phylogeny, and closely related species differed more than expected (K = 0.46, p = 0.03;

lambda = 0.06, p = 1), as did post-pelvic aspect ratio (K = 0.46, p = 0.02; lambda = 0.72, p = 0.1). Inspection of the phylogeny shows rheophilic genera such as *Myloplus, Tometes, Ossubtus*, and others, have either reduced or lost the anterior keel in its entirety. Loss or reduction of the keel was indicated by combinations of several factors: (1) the anterior keel is totally absent, (2) the length of the anterior keel is less than 150% the length of the pelvic girdle basal plate, and/or (3) anterior serrae show dramatic reductions in size and mineralization. In those taxa where the keel is reduced, conditions (2) and (3) are always coincident.

Asymmetry, injury, and aberrant serrae. In 42 of 61 specimens, we noticed aberrations, injuries, or asymmetry in serrae morphology (Fig. 6) summarized in Table 1. Aberrant serrae came in three varieties, (1) serrae asymmetry (flange loss), (2) serrae fusion, and (3) perforations. The first of these varieties, bilaterally asymmetrical serrae, is where individual serrae are lacking either a left or right lateral flange (Fig. 6). In these cases, an adjacent anterior or posterior serrae have a lateral flange which become enlarged to fill the gap formed by the missing flange. In the most extreme cases of flange loss, one of the lateral flanges have become entirely disconnected from surrounding skeleton (floating within connective tissue). Flange loss was less common than serrae fusion, seen in 30 of 68 or 44% of the specimens.

Some of these asymmetrical serrae are further modified, becoming fused with an adjacent serrae on the same side. Serrae fuse either of two ways, (1) when the paired lateral flanges of one serrae fuse to the paired flanges of an adjacent serrae (seemingly in absence of a cusp) or (2) where one of the lateral flanges is missing, and the remaining cusp and its singular flange fuse to both (anterior and posterior) flanking serrae. Serrae fusion frequently accompanied flange loss, but occurred more frequently in 51% of specimens (35 of 68).

Some serrae have perforations, circular holes which penetrate the entire thickness of the serrae (Fig. 6, *S. medinai*). This manner of injury was found least frequently, in only some 13% of serrasalmids examined (9 of 68). Similar perforations are evident elsewhere in the same specimens, noticeably on the opercular skeletal series (8% of specimens). Regardless of the nature of damaged serrae, damage to the keel is infrequently observed in the post-pelvic keel region (in 5 of 68, or 7.3% of specimens), with the only damage observed in this region found in the herbivorous species *Myleus setiger, Piaractus brachypomus, Mylossoma duriventre,* and *Colossoma macropomum.* Damage to the jaws was observed in nearly a quarter (24%) of specimens. Our survey of other characiforms for injuries to the skeleton found jaw damage in one specimen of *Hyphessobrycon anisitsi*, with no other damage detected in any individuals.

DISCUSSION

The frequency with which we observed damage to piranha skeletons was the highest we know of for actinopterygian fishes; the majority (67.6%) of serrasalmids had some form of skeletal damage (but see Reimchen, 1992). Serrasalmids appear to live violent lives, particularly in comparison to the 25 characiform specimens we surveyed. The keel was frequently damaged, with 57% bearing some aberration of the keel and the serrae. We suggest that damage to serrae is a sign of injury (in the case of asymmetrical serrae and perforations) and potential healing (i.e., serrae fusion).

We propose that interspecific (confamilial) and intraspecific interactions among serrasalmids drove the evolution of the armored keel. Armor in fishes has been attributed to defense against predation (Recher and Recher, 1968; Price et al., 2015), but armor can also be a defense against conspecifics (Huntingford, 1981). Most piranhas and pacus live in large schools, often in turbid water, and we suggest they use nipping as a form of communication with nearby individuals. The impressive teeth, set in a well-mineralized jaw, and powered by strong adductors (Grubich et al., 2012), means a gentle nip could lead to serious injury. This injury could be fatal in the belly region where the internal organs are not protected by ribs. The keel effectively covers this vulnerable area and takes considerable damage in wild fishes. Presence of the keel in all serrasalmids examined suggests that the ancestor of the family had a keel and that this structure was largely similar to both modern and extinct *Serrasalmus* species (Cione et al., 2009; Fig. 3D) and pacus (*Colossoma* and *Piaractus*), that is, a well-imbricated, flattened keel with both pre-pelvic and post-pelvic modules. Based on the presence of the keel in all serrasalmids we examined, we suggest this structure is a synapomorphy for the clade, and may stand as evidence of the early evolution of dense schooling in this group and their aggressive intraspecific interactions.

In this context, the prevalence of damage should be a function of aggregation size in piranhas and pacus. Sazima and Machado (1990) describe relative abundances and social behavior of three piranha species: *Serrasalmus marginatus, S. spilopleura,* and *Pygocentrus nattereri.* Our survey reveals no damage in *S. marginatus*, a solitary scale-feeder, while we observe some damage in *S. spilopleura* (2 of 6 types of damage), and the greatest damage prevalence in *P. nattereri* (5 of 6 types of damage), fishes which according to Sazima and Machado (1990) occur in aggregations of 5–6 individuals and 20–30 individuals, respectively. This argues in favor of more natural history observations on the behavior and autecology of piranha.

The serrae structure is the fundamental unit driving variation in overall keel morphology. Serrae vary among species and within keels themselves, with the disparity between pre-pelvic and post-pelvic serrae in one taxon occasionally trumping the variation of serrae within its genus. While some serrasalmids have pre-pelvic and post-pelvic serrae which are near identical, other taxa have pre-pelvic and post-pelvic serrae which differ considerably, from plate-like scutes to dagger-shaped stanchions. What the shape of individual serra confer defensively is difficult to disentangle from phylogeny; however, we observe that the widest serra are present in those species (namely *Pygocentrus*) with the largest gapes. Fin spines have been heralded as a means of making acanthopterygians unpalatable by artificially inflating their height to gape-limited predators (Price et al., 2015). Perhaps serrae shape is being influenced in a similar fashion, with tall or wide serrae corresponding to differences in attack mode between piranhas, which vary from head-on collisions (e.g., *Catoprion*), to lateral head-shaking (e.g., *Pygopristis*) (Sazima and Machado, 1990; Janovetz, 2005).

The stark reduction or total loss of the anterior keel in serrasalmids from swiftly flowing rivers, might suggest a hydrodynamic or locomotory function. Rheophilic serrasalmids also tend to have enlarged pelvic girdles and fins, presumably for increased stability in turbulent flow. The lack of an anterior keel segment in these species could allow broader muscle attachment areas for pelvic fins. But, rheophilic habitats are also more protected from predators (Lujan and Conway, 2015), and schooling is reduced. We do not know if competitive interactions between serrasalmids are reduced in these environments, but the large, predatory piranhas are residents of low-flow backwaters (Haddad and Sazima, 2003). The loss of anterior keel in high flow environments may reflect reduced intraspecific interaction rather than an adaptation to high flow.

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