DOI: 10.1111/ede.12306

RESEARCH

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Tooth and consequences: Heterodonty and dental replacement in piranhas and pacus (Serrasalmidae)

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Funding information
National Institutes of Health, Grant/Award Number: Pre-Doc 5-T32-GM07117 to FJI; National Science Foundation, Division of Biological Infrastructure, Grant/Award Number: 1712015 to MAK; Clyde and Lois Marlatt, Grant/Award Number: Fellowship to KEB; National Science Foundation, Grant/Award Numbers: BSR-85-14265 to FJI, NSF Graduate Research Fellowship to KEB

Abstract

Tooth replacement in piranhas is unusual: all teeth on one side of the head are lost as a unit, then replaced simultaneously. We used histology and microCT to examine tooth-replacement modes across carnivorous piranhas and their herbivorous pacu cousins (Serrasalmidae) and then mapped replacement patterns onto a molecular phylogeny. Pacu teeth develop and are replaced in a manner like piranhas. For serrasalmids, unilateral tooth replacement is not an "all or nothing" phenomenon; we demonstrate that both sides of the jaws have developing tooth rows within them, albeit with one side more mineralized than the other. All serrasalmids (except one) share unilateral tooth replacement, so this is not an adaptation for carnivory. All serrasalmids have interlocking teeth; piranhas interdigitate lateral tooth cusps with adjacent teeth, forming a singular saw-like blade, whereas lateral cusps in pacus clasp together. For serrasalmids to have an interlocking dentition, their teeth need to develop and erupt at the same time. We propose that interlocking mechanisms prevent tooth loss and ensure continued functionality of the feeding apparatus. Serrasalmid dentitions are ubiquitously heterodont, having incisiform and molariform dentitions reminiscent of mammals. Finally, we propose that simultaneous tooth replacement be considered as a synapomorphy for the family.

1 | INTRODUCTION

Piranhas (Serrasalmidae) feed on an anatomical potpourri of fish parts, ranging from scales and fin rays, chunks of flesh, and whole fishes (Northcote, Northcote, & Arcifa, 1986; Silva, Zina, Ferreira, Gomiero, & Goitein, 2015). These prey components are important at different ontogenetic stages; many juvenile piranhas (e.g., *Pygocentrus*, *Serrasalmus*, and *Pygopristis*) are facultative ectoparasites of other fishes (pterygophages and lepidophages), and then switch to outright piscivory or carnivory as subadults (Sazima & Machado, 1990; Nico & Taphorn, 1988). Correspondingly, piranha dentitions are razor-sharp and continuously replaced (polyphyodont): All teeth on one side of the head are lost and then replaced simultaneously (unilateral replacement; Berkovitz, 1975; Roberts, 1967) to maintain a continuously sharp edge for feeding on flesh (Berkovitz & Shellis, 1978; Shellis & Berkovitz, 1976). However, carnivorous piranhas stem from ancestors they share with their cousins, the herbivorous pacus, together comprising the family Serrasalmidae (Ortí, Sivasundar, Dietz, & Jégu, 2008;

Thompson, Betancur-R, López-Fernández, & Ortí, 2014). In contrast to piranhas, pacus (e.g., *Colossoma*, *Myloplus*, *Piaractus*, etc.) and pacucitos (e.g., *Metynnis*) have complex dentitions with molariform teeth and feed on a wide variety of prey, including aquatic grasses, seeds, nuts, and whole fruits (Andrade, Giarrizzo, & Jegu, 2013; Correa, Winemiller, Lopez-Fernandez, & Galetti, 2007; Figure 1). This raises the question of whether unilateral tooth replacement in piranhas is restricted to carnivores or is widespread among all serrasalmids.

Serrasalmid teeth are firmly ankylosed to the jaws via collagen and unmineralized, cancellous bone (Shellis & Berkovitz, 1976), rendering the entire dental battery immobile (Shellis & Berkovitz, 1976); the skull is also largely akinetic due to reduction of the maxilla or even its fusion with the premaxilla (Alexander, 1964, 1975). In piranhas, the teeth also interlock, with accessory cusps of a preceding tooth fitting into a recess on the anterior, adjacent tooth (Shellis & Berkovitz, 1976). Whether pacu teeth lock together is currently unknown. Replacement teeth develop underneath the functional tooth row intraosseously within a "crypt" or bony chamber within the jaws (Berkovitz, 1975; Shellis & Berkovitz, 1976; Figure 2). Once fully mineralized, teeth rise from the crypt, requiring drastic internal remodeling of the jaws as the teeth ascend through cancellous bone. During the latency period between when functional teeth are lost and new teeth become active, piranhas are limited to feeding with only one side of their jaws (Shellis & Berkovitz, 1976), presumably gumming their prey or not feeding in the interim (Berkovitz & Shellis, 1978). This turnover between functional teeth and their underlying replacement dentition has never been directly observed, but is presumably rapid (Berkovitz, 1975, 1980; Berkovitz & Shellis, 1978; Shellis & Berkovitz, 1976).

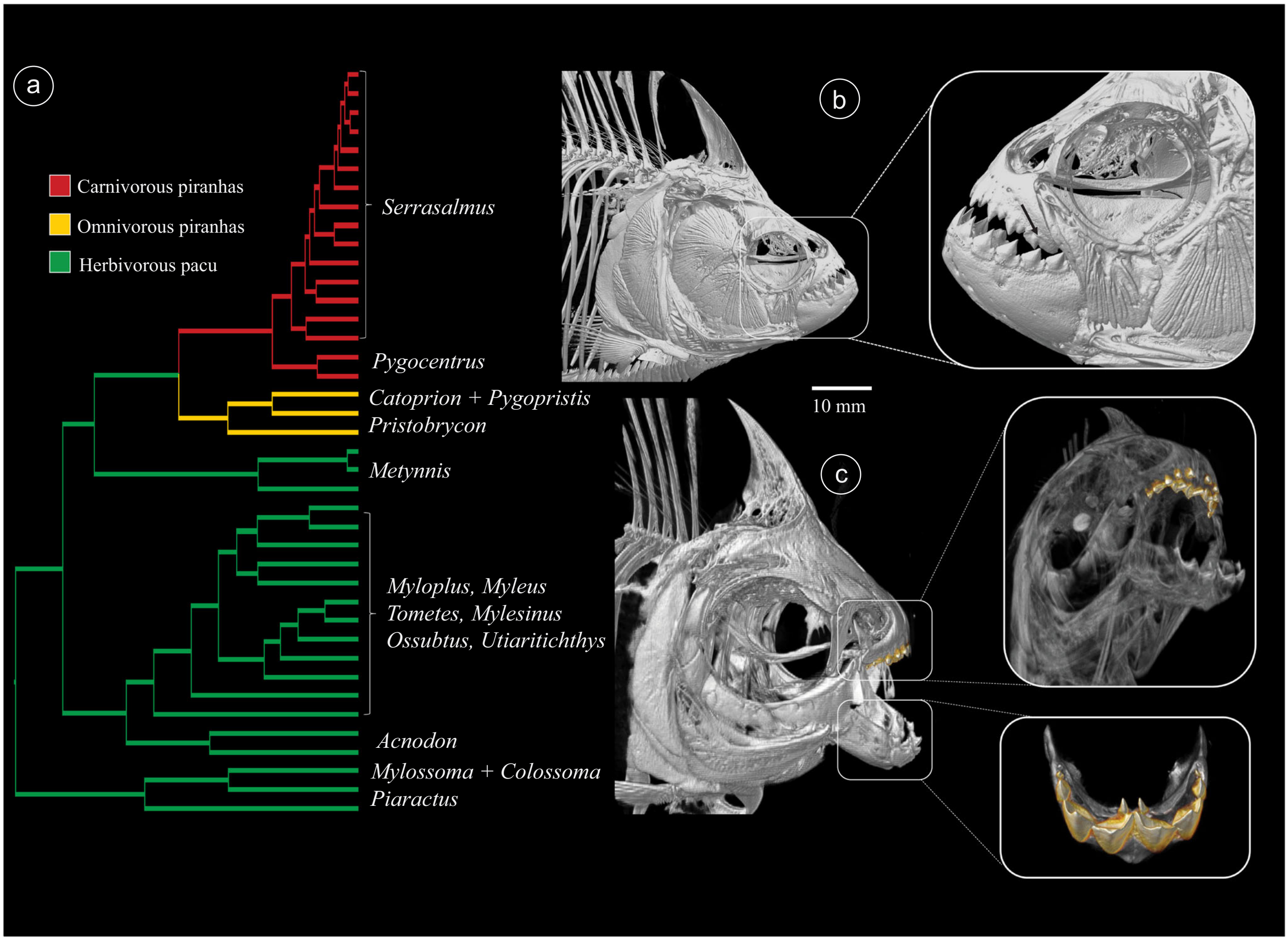


FIGURE 1 Examples of heterodonty in serrasalmids and phylogeny used in this study. (a) currently accepted hypothesis of serrasalmid relationships from Thompson et al. (2014). (b) Heterodont dentitions in carnivorous *Serrasalmus altuvei* (UMMZ 215066; 150 mm, from left to right—anterior and lateral orientations); note the carnassial-like posterior-most teeth shown circled in the inset. (c) Teeth in herbivorous *Myloplus rubripinnis* (ANSP 199578; 120 mm, tooth rows are false colored in orange. Ventral view of the premaxillary teeth (upper inset), note the labial and lingual tooth rows. Dorsal view of the dentary angled anteriorly [Color figure can be viewed at wileyonlinelibrary.com]

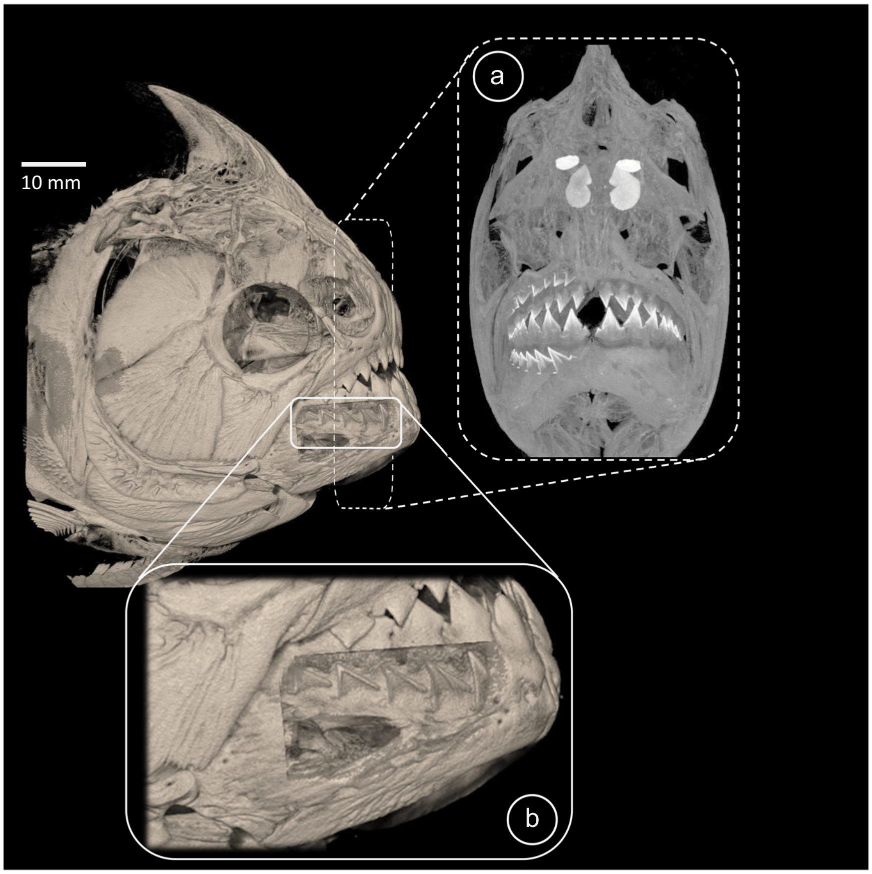


FIGURE 2 Rendered computed tomography scan of *Pygocentrus nattereri* ROM 85941 (170 mm SL). Dotted inset (a) shows a transverse section through the skull using the maximum intensity projection setting, with evident replacement teeth developing in the right-side crypt. Inset (b) shows a sagittal section through the crypt with developing replacement teeth [Color figure can be viewed at wileyonlinelibrary.com]

Serrasalmids also have conspicuously heterodont dentitions (Alexander, 1964; Roberts, 1967). In fact, the arrangement and shape of teeth in pacus is the most obvious means of distinguishing between many genera (e.g., *Tometes*, *Myleus*, *Mylesinus*, *Myloplus*, *Ossubtus*, and *Utiaritichthys*; Jégu, 1992; Andrade et al., 2013; Andrade, Jégu, & Giarrizzo, 2016; Pereira & Castro, 2014). In many pacus, anterior, incisiform teeth-crop vegetation, which is then processed by adjacent, usually posterior teeth with broader cusps; in effect, mirroring the processing of many grazing mammals (Alexander, 1964). Piranhas also display some degree of heterodonty, as the posterior-most premaxillary tooth is stouter and longer than other teeth, resembling a mammalian carnassial (Alexander, 1964). Simultaneous tooth replacement in serrasalmids might then be explained by either of two hypotheses: (a) that saw-like dental batteries in piranhas require unilateral replacement to maintain a sharp cutting edge (Berkovitz, 1975), whereas dentitions with other functions like fracturing seeds or dislodging scales may be less constrained, or (b) that constructional constraints on replacing large, interlocking, heterodont dentitions require that all teeth must be replaced simultaneously, rather than in a piecemeal fashion.

Whether pacus (Figure 1), share unilateral simultaneous tooth replacement with piranhas, should illuminate whether unilateral tooth replacement is either a novel strategy for carnivory (in piranhas) or an exaptation for such (and is ubiquitous among serrasalmids). Similarly, whether interlocking teeth are an autapomorphy for piranhas or something widespread among serrasalmids, should also inform whether this trait is tied to either dietary ecology or the symptom of some developmental process. We visualized and described the patterns of tooth development and replacement in both a piranha (*Pygocentrus*) and related pacus (*Metynnis* and *Myloplus*) using histological staining and sectioning. We then expanded the taxonomic scope of our study by using microcomputed tomography (μCT) scanning, histological sectioning, light microscopy, and scanning electron microscopy (SEM) to survey whether asymmetrical patterns of tooth replacement, heterodonty, and interlocking teeth are common across all serrasalmids or present only in piranhas. The goals of this project were threefold: (a) characterize tooth-replacement patterns in piranhas and pacus, using *Pygocentrus*, *Metynnis*, and *Myloplus* as models, (b) document tooth-replacement strategies across serrasalmids using both μCT and histology, and (c) explore the evolution of tooth replacement, interlocking dentitions, and heterodonty across serrasalmids and the systematic importance of these characters. We expect that pacus and piranhas will share a simultaneous tooth-replacement mode, which was then exapted for carnivory in piranhas.

2 | METHODS

2.1 | Histological preparation, staining, and visualization

We used deceased, wild-caught specimens obtained from the aquarium trade (Rio Nanay, Peru) of the piranha *Pygocentrus nattereri* ($n = 7$; 32–102 mm SL) and pacucitos *Metynnis maculatus* ($n = 6$; 29.5–52.2 mm SL), as well as single specimens of the pacus *Myloplus rubripinnis* (50 mm SL) and *Myloplus schomburgkii* (52 mm SL). Samples were shipped from suppliers frozen and thawed before collection of tissues for histological staining. We removed the upper and lower jaws from each specimen and fixed them in a 10% buffered formalin solution for 48 hr. Samples were then rinsed in dH_2O and decalcified following the protocol laid out in the Histological Processing of Teeth and Periodontal Tissues for Light Microscopy Analysis (Silva, Moreira, & Alves, 2011) and then placed in 10% ethylenediaminetetraacetic acid for 9 days. After tissues were fully decalcified, we processed samples using a stepwise dehydration series. We split the upper and lower jaws in half at the medial symphysis of the dentary or premaxillae, and then separately embedded each ramus in JB-4 following the Electron Microscopy Science JB-4 embedding media protocol. Embedded specimens were sectioned parasagittally and transversally at 3–3.5 μm. Finally, we placed sections onto glass slides, dried them for 24 hr, and then stained slides with Lee's methylene blue-basic fuchsin stain.

2.2 | Microcomputed tomography scanning

Our study includes 93 specimens from all 16 extant genera including 40 species, over one-third of total serrasalmid taxonomic diversity (Table 1). Specimens were obtained from museums institutions, including the Burke Museum (UW, Seattle, WA), Field Museum of Natural History (FMNH; Chicago, IL), Academy of Natural Sciences of Drexel University (ANSP; Philadelphia, PA), California Academy of Sciences (CAS; San Francisco, CA), Texas A&M Biodiversity and Research Collections (TCWC; Galveston, TX), Auburn University Museum (AUM; Auburn, AL), the Smithsonian National Museum of Natural History (NMNH), and Royal Ontario Museum (ROM; Toronto, ON, Canada). All data are freely available via the #ScanAllFishes project database on Open Science Framework and MorphoSource (morphosource.org).

Specimens were μCT-scanned using the Bruker 1173 SkyScan (Micro Photonics Inc., Allentown, PA) at Friday Harbor Laboratories' Karel Liem Memorial Bio-Imaging Facility. Before μCT scanning, specimens were photographed with an individual radio-opaque label and scale bar, then wrapped in ethanol-soaked cheesecloth to prevent drying. The wrapped specimens were packed as a group into 3D-printed (polyvinyl alcohol) plastic tubes, and then sealed with plastic wrap to limit desiccation. Scans were typically run at 65 kV and 123 μA, with voxel sizes 17.4–50.0 μm, and reconstructed as .bmp image stacks. Specimens were digitally isolated using Dataviewer software (2012–15 Bruker microCT, Dataviewer Version 1.5.24, 64-bit) and converted to .dcm format using DicomConverter software (2007–17 Bruker microCT, DicomCT, Version 2.5).

2.3 | Scanning electron microscopy

We used SEM to study tooth-interlocking mechanisms and explore the morphology of teeth in greater detail. Whole jaws and attached teeth were excised from specimens and sections were gradually dehydrated from 70 to 100% EtOH over 48 hr. Samples were critical-point dried (Samdri 790, Tousimis Research Corp., Rockville, MD), sputter-coated with gold (SPI Sputter 12121, SPI Supplies/Structure Pro, West Chester, PA), and imaged using SEM (Neoscope JCM-5000, Tokyo, Japan). We photographed the microstructure of both the upper and lower jaws of *Colossoma macropomum* MCZ 19003 ($n = 2$, 125–168 mm SL), *Myloplus schomburgkii* ($n = 2$, 52–60 mm SL), *Metynnis lippincottianus* MCZ 36956 ($n = 8$, 96–120 mm SL), *Pristobrycon striolatus* MCZ 19260 ($n = 2$, 110–115 mm SL), and *Catoprion mento* ($n = 2$, 45–53 mm SL).

2.4 | Character scoring & comparative methods

We used SEM, CT, histology, and light microscopy to confirm aspects of tooth-interlocking and replacement mechanisms. We used the maximum intensity projection imaging function of Bruker CTVox volume-render software to more clearly observe the densest tissues (i.e., teeth and otoliths), relative to the rest of the skeleton (Figure 2). Typically, on one side of the skull where teeth were being replaced, we would find two functional rows of teeth (upper and lower) situated over well-developed and mineralized replacement teeth in the crypt. On the opposite side of the skull, we would also find two functional rows of teeth on the upper and lower jaw and either no evidence of teeth developing in the crypt or a less-mineralized set of replacement teeth, relative to the opposite set. Such a scenario was considered an example of unilateral replacement when the developing tooth row was present only on one side of the jaws or when one set was much more developed (mineralized) than the other. If either scenario was not satisfied, we scored the taxa as "indeterminate."

TABLE 1 List of specimens, their associated museum collections, and scored table of tooth replacement and heterodonty

Genus species	Museum	Catalog no.	Replacement mode	Upper jaw heterodonty	Lower jaw heterodonty
Colossoma macropomum	SU-CAS	55894	Unilateral	Monognathic	Monognathic
Colossoma macropomum	SU-CAS	55894	Unilateral	Monognathic	Monognathic
Colossoma macropomum	FMNH	78087	Unilateral	Monognathic	Monognathic
Colossoma macropomum	FMNH	78087	Unilateral	Monognathic	Monognathic
Colossoma macropomum	FMNH	78087	Unilateral	Monognathic	Monognathic
Colossoma macropomum	CAS	69233	Unilateral	Monognathic	Monognathic
Mylossoma duriventre	CAS	15675	Unilateral	Monognathic	Monognathic
Mylossoma duriventre	CAS	15675	Unilateral	Monognathic	Monognathic
Mylossoma duriventre	CAS	15675	Bilateral	Monognathic	Monognathic
Mylossoma duriventre	CAS	15675	Indeterminate	Monognathic	Monognathic
Mylossoma duriventre	SU-CAS	54683	Unilateral	Monognathic	Monognathic
Mylossoma duriventre	SU-CAS	54683	Unilateral	Monognathic	Monognathic
Mylossoma duriventre	CAS	54683	Unilateral	Monognathic	Monognathic
Piaractus brachypomus	ANSP	166685	Unilateral	Monognathic	Monognathic
Piaractus brachypomus	ANSP	8023	Unilateral	Monognathic	Monognathic
Acnodon oligacanthus	ROM	100851	Unilateral	Monognathic	Monognathic
Acnodon oligacanthus	ROM	100851	Unilateral	Monognathic	Monognathic
Acnodon oligacanthus	ROM	100842	Unilateral	Monognathic	Monognathic
Acnodon oligacanthus	ROM	100842	unilateral	Monognathic	Monognathic
Acnodon normani	CAS	20739	Bilateral	Monognathic	Monognathic
Acnodon normani	ANSP	199545	Unilateral	Monognathic	Monognathic
Acnodon normani	ANSP	199545	Unilateral	Monognathic	Monognathic
Mylesinus paucisquamatus	CAS	20221	Unilateral	Dignathic	Monognathic
Myleus setiger	ANSP	197912	Unilateral	Monognathic	Monognathic
Myleus micans	ANSP	171893	Unilateral	Monognathic	Monognathic
Myleus micans	ANSP	171893	Unilateral	Monognathic	Monognathic
Myloplus schomburgkii	SU-CAS	70039	Unilateral	Monognathic	Monognathic
Myloplus rhomboidalis	ROM	86133	Unilateral	Monognathic	Monognathic
Myloplus rhomboidalis	SU-CAS	34504	Unilateral	Monognathic	Monognathic
Myloplus rubripinnis	ANSP	199578	Unilateral	Monognathic	Monognathic
Myloplus rubripinnis	TCWC	8411.02	Unilateral	Monognathic	Monognathic
Myloplus levis	FMNH	56928	Unilateral	Monognathic	Monognathic
Myloplus asterias	FMNH	69916	Unilateral	Monognathic	Monognathic
Myloplus asterias	FMNH	69916	Unilateral	Monognathic	Monognathic
Myloplus torquatus	FMNH	109794	Unilateral	Monognathic	Monognathic
Metynnis luna	CAS	11770	Unilateral	Monognathic	Monognathic
Metynnis hypsauchen	SU-CAS	59384	Unilateral	Indeterminate	Indeterminate
Metynnis hypsauchen	FMNH	56916	Indeterminate	Monognathic	Monognathic
Metynnis argenteus	AUM	22490	Unilateral	Monognathic	Monognathic
Tometes trilobatus	ROM	103700	Unilateral	Monognathic	Monognathic
Tometes trilobatus	ROM	103700	Unilateral	Monognathic	Monognathic
Tometes kranponhah	ANSP	196744	Unilateral	Monognathic	Monognathic
Tometes kranponhah	ANSP	196745	Unilateral	Monognathic	Monognathic
Tometes kranponhah	ANSP	196745	Unilateral	Monognathic	Monognathic

(Continues)

TABLE 1 (Continued)

Genus species	Museum	Catalog no.	Replacement mode	Upper jaw heterodonty	Lower jaw heterodonty
Ossubtus xinguense	ANSP	197392	Unilateral	Monognathic	Monognathic
Ossubtus xinguense	ANSP	194758	Unilateral	Monognathic	Monognathic
Utiaritichthys sp.	ANSP	180811	Unilateral	Monognathic	Monognathic
Catoprion mento	CAS	20216	Bilateral	Monognathic	Monognathic
Catoprion mento	CAS	20216	Bilateral	Monognathic	Monognathic
Catoprion mento	ROM	95239	Bilateral	Monognathic	Monognathic
Pristobrycon striolatus	ANSP	166906	Unilateral	Monognathic	Monognathic
Pygopristis denticulata	CAS	20208	Unilateral	Monognathic	Monognathic
Pygopristis denticulata	AUM	36164	Unilateral	Monognathic	Monognathic
Pygopristis denticulata	AUM	36164	Unilateral	Monognathic	Monognathic
Pygopristis denticulata	AUM	36164	Unilateral	Monognathic	Monognathic
Pristobrycon calmoni	CAS	15277	Unilateral	Monognathic	Monognathic
Pristobrycon calmoni	CAS	15277	Unilateral	Monognathic	Monognathic
Serrasalmus altipinnis	ROM	85776	Unilateral	Monognathic	Monognathic
Serrasalmus eigenmanni	SU-CAS	21982	Unilateral	Monognathic	Monognathic
Serrasalmus eigenmanni	ROM	97245	Unilateral	Monognathic	Monognathic
Serrasalmus eigenmanni	ROM	97246	Unilateral	Monognathic	Monognathic
Serrasalmus manueli	ANSP	198551	Bilateral	Monognathic	Monognathic
Serrasalmus medinai	AUM	43322	Unilateral	Monognathic	Monognathic
Serrasalmus medinai	AUM	43326	Unilateral	Monognathic	Monognathic
Serrasalmus medinai	AUM	53950	Unilateral	Monognathic	Monognathic
Serrasalmus medinai	ANSP	165387	Bilateral	Monognathic	Monognathic
Serrasalmus medinai	TCWC	7503.06	Unilateral	Monognathic	Monognathic
Serrasalmus spilopleura	SU-CAS	16773	Unilateral	Monognathic	Monognathic
Serrasalmus spilopleura	FMNH	108506	Unilateral	Monognathic	Monognathic
Serrasalmus spilopleura	FMNH	108506	Unilateral	Monognathic	Monognathic
Serrasalmus irritans	FMNH	100130	Unilateral	Monognathic	Monognathic
Serrasalmus irritans	FMNH	100130	Unilateral	Monognathic	Monognathic
Serrasalmus brandtii	FMNH	56945	Unilateral	Monognathic	Monognathic
Serrasalmus brandtii	FMNH	56945	Unilateral	Monognathic	Monognathic
Serrasalmus maculatus	FMNH	56976	Indeterminate	Monognathic	Monognathic
Serrasalmus maculatus	FMNH	56976	Unilateral	Monognathic	Monognathic
Serrasalmus rhombeus	FMNH	111315	Unilateral	Monognathic	Monognathic
Serrasalmus rhombeus	FMNH	111315	Unilateral	Monognathic	Monognathic
Serrasalmus marginatus	FMNH	71030	Unilateral	Monognathic	Monognathic
Serrasalmus elongatus	FMNH	111314	Unilateral	Monognathic	Monognathic
Serrasalmus elongatus	FMNH	111314	Unilateral	Monognathic	Monognathic
Serrasalmus humeralis	FMNH	56969	Unilateral	Monognathic	Monognathic
Serrasalmus humeralis	FMNH	56969	Indeterminate	Monognathic	Monognathic
Serrasalmus humeralis	ANSP	188683	Unilateral	Monognathic	Monognathic
Serrasalmus humeralis	ANSP	188683	Unilateral	Monognathic	monognathic
Pygocentrus cariba	FMNH	103670	Unilateral	Monognathic	Monognathic
Pygocentrus cariba	FMNH	103670	Unilateral	Monognathic	Monognathic
Pygocentrus nattereri	FMNH	111306	Unilateral	Monognathic	Monognathic
Pygocentrus nattereri	FMNH	111306	Indeterminate	Monognathic	Monognathic

(Continues)

TABLE 1 (Continued)

Genus species	Museum	Catalog no.	Replacement mode	Upper jaw heterodonty	Lower jaw heterodonty
Pygocentrus nattereri	FMNH	111306	Unilateral	Monognathic	Monognathic
Pygocentrus nattereri	CAS	71016	Unilateral	Monognathic	Monognathic
Pygocentrus piraya	CAS	71018	Unilateral	Monognathic	Monognathic
Pygocentrus piraya	CAS	71018	Unilateral	Monognathic	Monognathic

Abbreviations: ANSP, Academy of Natural Sciences, Philadelphia; AUM, Auburn University Museum; FMNH, Field Museum of Natural History; ROM, Royal Ontario Museum; SU-CAS/CAS, California Academy of Sciences; TCWC, Texas A&M Biodiversity and Research Collections.

To estimate the number of times different tooth-replacement modes have evolved across serrasalmid history, and if lineages have lost or modified these modes, we used the most current phylogenetic tree for serrasalmids (Thompson et al., 2014), *sans Utiaritichthys*, which is not present in any well-resolved phylogeny to date. We trimmed this tree to match our taxon sampling using the [drop.tip] function in R. We used Bayesian stochastic character mapping (Bollback, 2006; Huelsenbeck, Nielsen, & Bollback, 2003) and maximum likelihood-based means of ancestral state reconstruction to map changes in character states across the tree. Stochastic character mapping simulates histories of discrete traits over all branches of the tree, allowing evolutionary transitions to occur along the branches, whereas the maximum likelihood method we also used reconstructs ancestral states only at the nodes. We assumed equal rates for the evolutionary rate of transition from states and iterated this process 1000 times to determine the most stable set of reconstructed scenarios using the [make.simmap] function (*phytools*; Revell, 2012), and reconstruction at nodes using the [ace] function in *geiger* (Harmon, Weir, Brock, Glor, & Challenger, 2007). We used tests for phylogenetic signal to look for relationships between replacement pattern and phylogeny, implemented using the [phylosig] command in the *geiger* package. Finally, we defined heterodonty as differences in tooth size (size heterodonty) or tooth shape (shape heterodonty) along an individual jaw. Heterodont dentitions can be monognathic, where teeth vary in form along a jaw, as well as dignathic, where tooth form differs between the upper and lower jaws. We assessed heterodonty and surveyed its presence among serrasalmid taxa (Table 1). All analyses were performed in the R statistical programming environment (www.r-project.org).

3 | RESULTS

3.1 | Tooth development and replacement in serrasalmids

Tooth replacement proceeds similarly in both *Pygocentrus* (Figure 3) and *Metynnis*, whereby the dentition on one side of the jaws is replaced simultaneously as a unit, across all sizes of these fishes we examined. We observed similar patterns of tooth replacement regardless of ontogenetic stage, corroborating what others have found (Shellis & Berkovitz, 1976) and so we present data for fishes spanning size ranges. Given that we could not raise specimens or induce tooth replacement or certain stages of replacement, we instead surveyed replacement patterns across many individuals and compared tooth germ development between the right and left sides of skull. A newly germinating tooth bud is present soon after replacement of the functional row within the cancellous bone of the crypt. Teeth within a dental battery develop along an anterior–posterior axis, meaning that anterior teeth are more mineralized or “mature” than posterior teeth (Figure 4). However, at time of replacement the entire replacement row is well mineralized and emerging from their nascent tooth crypts (i.e., an “incoming” tooth *sensu* Bemis, Giuliano, & McGuire, 2005). Histological staining shows that teeth are developing on both sides of the jaws (Figure 5b) that were typically not detectable by CT scanning or radiographs. However, one side of the dentition is considerably more developed and more mineralized than the other; this more-mineralized replacement dentition is beneath an older functional tooth row whereas the less-mineralized side is under a more recently replaced dentition. We will continue to refer to this mode of tooth replacement as “unilateral” as one side of the replacement dentition is more developed than the opposite side.

In some specimens the residual tooth crypt space of the now-functional row is retained throughout the development of the nascent replacement dentition. As tooth germs increase in size, the cancellous bone forming the lateral boundaries of the crypt is gradually resorbed to make room for the nascent dentition. In later stages of tooth development, the cancellous bone underlying the functional teeth is similarly resorbed, and the internal pulp cavity of the functional tooth empties and becomes mostly hollow (i.e., “eroding” tooth *sensu* Bemis et al., 2005). The rising replacement teeth extend into the internal cavity formed by flanking cancellous bone of the jaws and “capped” by the interior of the functional tooth (Figure 4). Unlike piranhas, which have singular tooth rows on the upper and lower jaws, pacus generally have two distinct

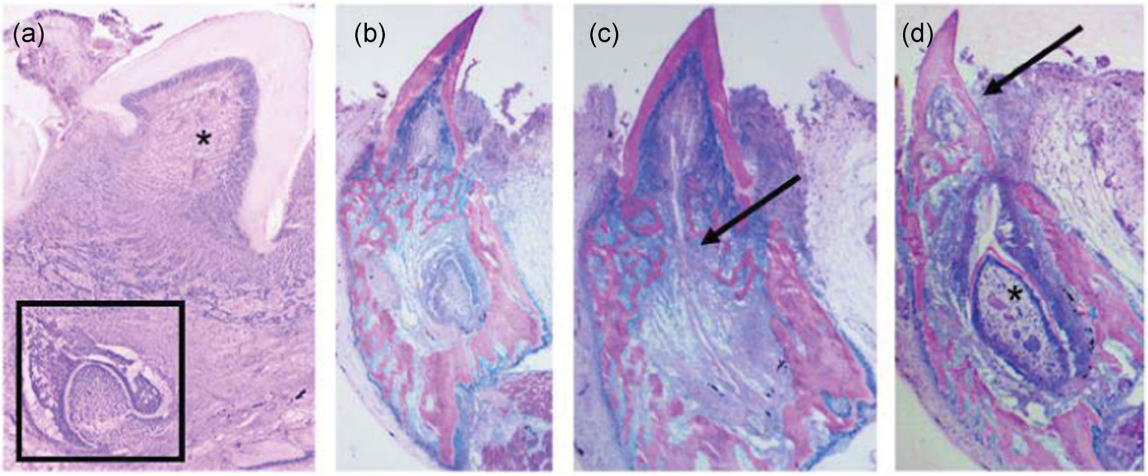


FIGURE 3 Developing tooth replacement in juvenile *Pygocentrus nattereri* specimens (90 mm SL). (a) Newly replaced tooth with small germinating tooth (box) inside of the bony crypt. Note the multiple layers within the dentine of the functional tooth (asterisk). (b) Functioning tooth and developing replacement tooth have become more robust. (c) Draining of the functional tooth internal pulp into the bony crypt (arrow). (d) Pulp material from the functional tooth is drained or resorbed, with only outer dentine layer left (arrow); replacement tooth is fully mineralized (asterisk) [Color figure can be viewed at wileyonlinelibrary.com]

tooth rows in both the upper and lower jaws (except *Myloplus ternetzi*, which lack lingual dentary teeth; Supporting Information). In both *Metynnis* and *Myloplus,* these lingual and labial tooth rows are replaced simultaneously, and the nascent dentition is supported by a similar cohort of tissues (Figure 5), albeit with less-connective tissue than piranhas (Figure 4). The replacement dentition is primarily supported by dense irregular-connective and adipose tissues, until the teeth emerge fully mineralized and supported by newly formed cancellous bone ("incoming" tooth *sensu* Bemis et al., 2005). Developing teeth are not oriented in the same plane as the functional row until late in replacement, when they rotate rostrally before interlocking with adjacent teeth in the dentition (Figure 6). In *Metynnis*, developing teeth also form at an angle not consistent with the functional teeth and like piranhas, the teeth reorient shortly before replacement (see Trapani, 2001).

3.2 | Tooth-interlocking mechanisms

All serrasalmids interlock or buttress their teeth together, although there is considerable variation in how this occurs and whether either or both jaws have

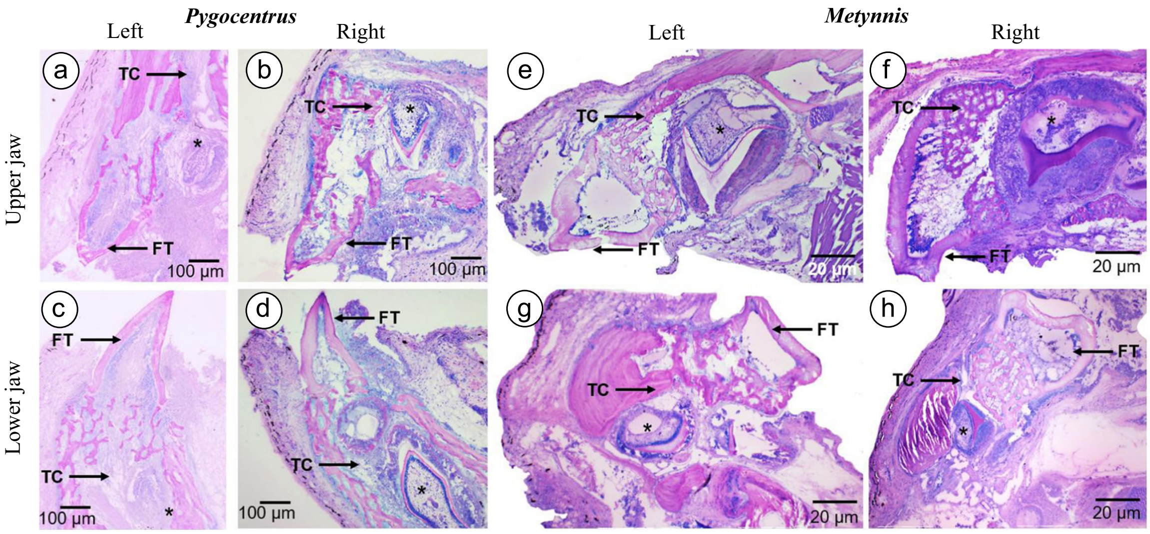
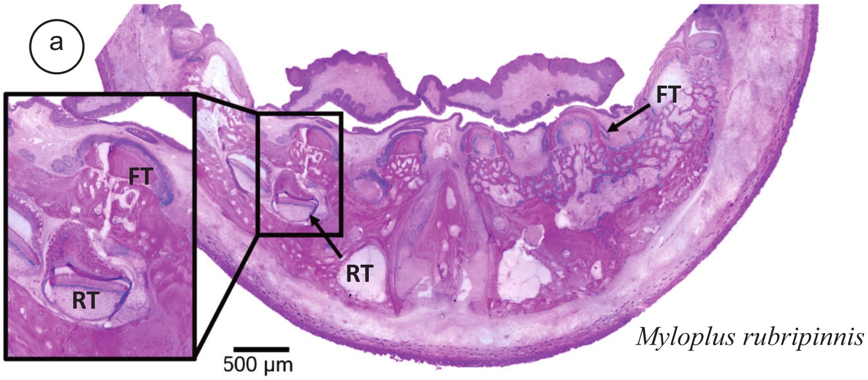


FIGURE 4 Histological sections showing unilateral tooth replacement of serrasalmid dentitions. (a–d) Parasagittal sections of upper and lower *Pygocentrus nattereri* jaws (90 mm SL) showing the functional and replacement tooth (asterisk) inside of the jaw crypt. (e–h) parasagittal sections of upper and lower *Metynnis maculatus* jaws (65 mm SL) showing the functional and replacement tooth (asterisk) inside of the jaw crypt. FT, functional tooth; TC, tooth crypt [Color figure can be viewed at wileyonlinelibrary.com]



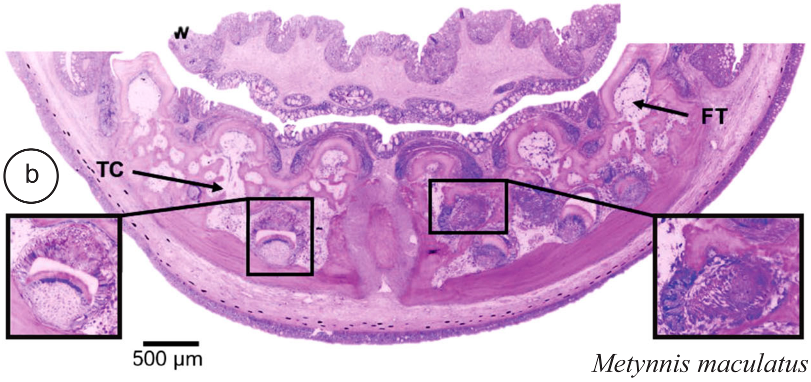


FIGURE 5 Bilateral tooth replacement among different pacu species. Images are frontal sections through the lower jaw of (a) *Myloplus rubripinnis* (50 mm SL) and (b) *Metynnis maculatus* (65 mm SL). In *Metynnis*, note the mature developing replacement tooth (left) and nascent replacement tooth (right) [Color figure can be viewed at wileyonlinelibrary.com]

interlocking dentitions (piranhas) or whether interlocking occurs only in the lower jaw (most pacus; Figure 7). Using SEM, we found the lower jaw teeth in *Colossoma* do not interlock together, but one or two teeth buttress one another (Figure 8). In *Myloplus* (*M. schomburgkii*), using SEM, we confirmed more rigorous buttressing among several of the lower jaw teeth, notably the symphyseal teeth with their nearest lateral neighbor (Figures 8 and 9); however, the upper jaw teeth do not meet. *Acnodon* was the only exception to this rule, with interlocking occurring in both premaxillary and dentary teeth, albeit sparsely in the former. In *Metynnis* (*M. lippincottianus*), we found that the lower jaw teeth interlock via clasp-like lateral extensions of the tooth margins whereas in the upper jaw only the most-posterior teeth appear to interlock (Figure 8).

In piranhas, the interlocking mechanism is radically different from pacus, and adjacent teeth in both premaxillary and dentary rows interlock intimately via a “peg and socket” mechanism. This mechanism is demonstrated in

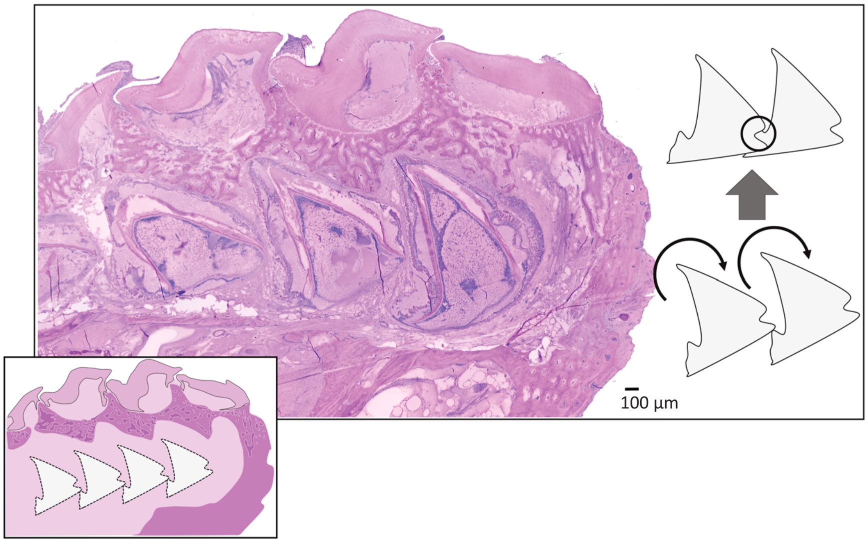


FIGURE 6 Development of replacement tooth orientation and interlocking mechanisms in a sagittal section through juvenile *Pygocentrus nattereri* right-side jaw (120 mm SL). Inset diagram (lower left) shows replacement teeth askew, before rotating into their interlocking (right) confirmation and elevating into functional position [Color figure can be viewed at wileyonlinelibrary.com]

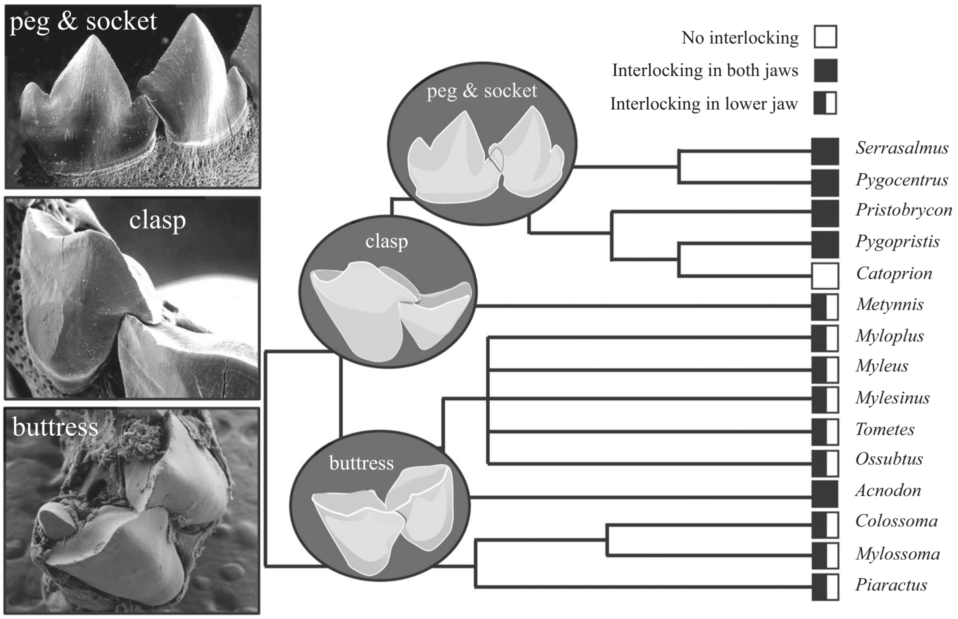


FIGURE 7 Distribution of tooth-interlocking mechanisms across serrasalmids. "Peg & socket" interlocking mechanism scanning electron microscopy (SEM) image from *Pristobrycon striolatus* MCZ 19260 (100–110 mm SL); "clasp" interlocking mechanism SEM from *Metynnis lippincottianus* MCZ 36956 (96–120 mm SL); "buttress" interlocking mechanism SEM from *Myloplus schomburgkii* (52 mm SL). In general, piranhas (*Pygopristis*, *Pristobrycon*, *Pygocentrus*, and *Serrasalmus*) have interlocking teeth with a peg & socket mechanism in both the upper and lower jaws. *Metynnis* also have strongly interlocking teeth ("clasp" mechanism), but only in the lower jaws. Larger pacu have more buttressed interlocking mechanisms present only teeth in the lower jaw (except in *Acnodon*) (Leite & Jegu, 1990)

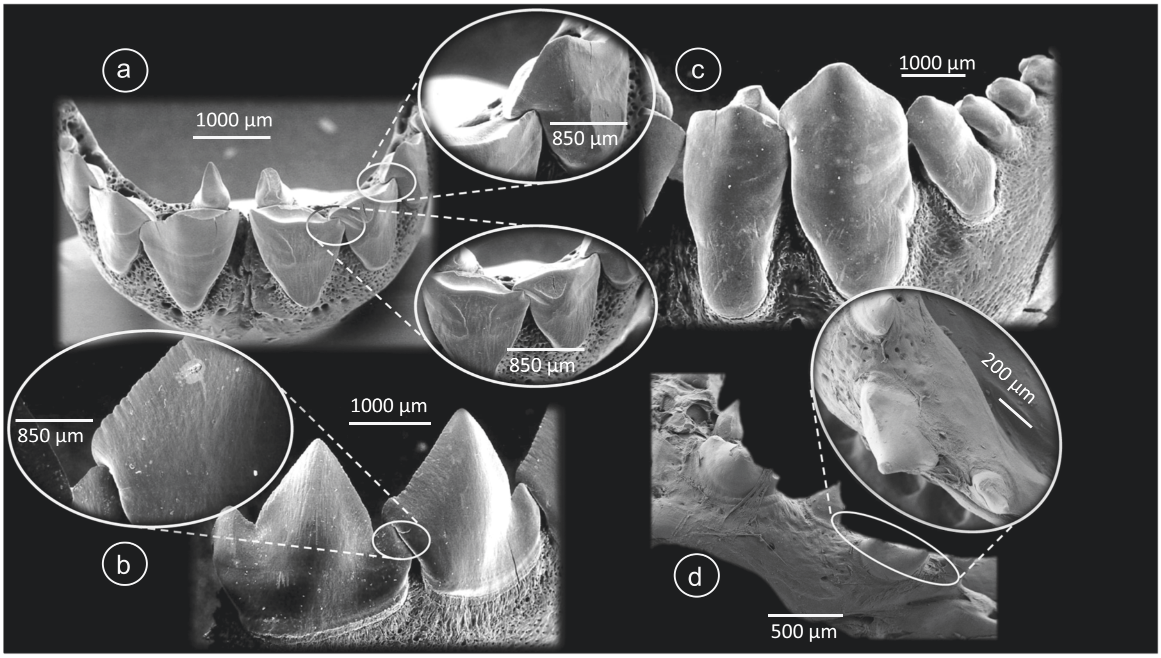


FIGURE 8 Scanning electron microscopy images contrasting tooth shape and interlocking mechanisms in serrasalmids. (a) *Metynnis lippincottianus* (MCZ 36956) lower jaw with inset images of interlocking mechanism, (b) *Pristobrycon striolatus* (MCZ 19260) teeth with inset images of cusp-interlocking mechanism, (c) *Colossoma macropomum* (MCZ 19003), lower jaw near symphysis with buttressing between symphyseal and adjacent (second) tooth, (d) *Catoprion mento* (53 mm SL), left view of lower jaw (jaw symphysis to the left) showing staggered spacing of teeth (noninterlocking)

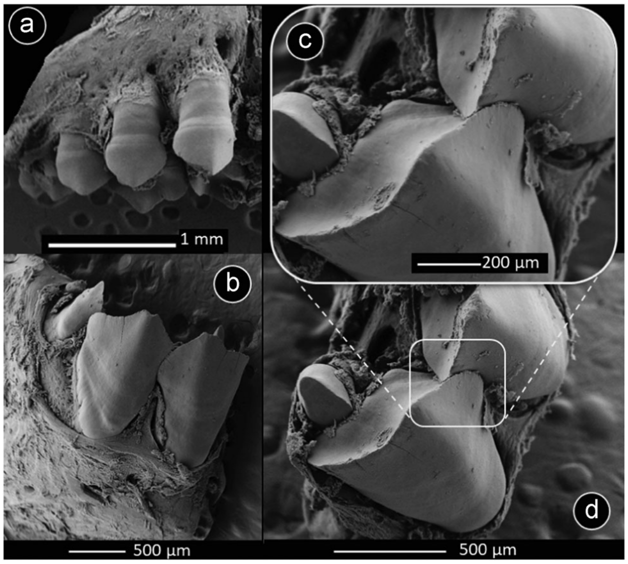


FIGURE 9 Scanning electron microscopy images of tooth shape and "clasp" interlocking mechanism in *Myloplus schomburgkii* (50 mm SL). (a) Upper jaw, with no visible interlocking mechanisms. (b) Lower jaw teeth with interlocking mechanism between symphyseal and adjacent teeth. (c) Close-up of interlocking "clasp" mechanism of lower jaw teeth. (d) Dorsal view of interlocking mechanism of symphyseal tooth and adjacent neighbor

Pristobrycon striolatus, where teeth interlocked via small lateral cusps that insert into spaces on adjacent teeth (Figure 8). Our histological sections also show evidence of tooth interlocking via lateral cusps in *Pygocentrus nattereri*, the teeth firmly interlock in both upper and lower jaws via small lateral cusps that insert into space on adjacent teeth. Finally, Shellis and Berkovitz (1976) report on interlocking teeth in *Serrasalmus rhombeus*, confirming the presence of such mechanisms (also via lateral cusps) in this genus. However, the scale-feeding piranha *Catoprion* lacks any interlocking mechanism between teeth in either the upper or lower jaw (Figure 8).

3.3 | Tooth morphology across serrasalmid genera

Most pacus (*Myleus*, *Myloplus*, *Tometes*, *Utiaritichthys*, *Mylossoma*, *Colossoma*, and *Piaractus*) and pacucitos (*Metynnis*) have two rows of teeth on the upper and lower jaws, excluding *Acnodon* and *Mylesinus*, whereas piranhas have single rows of premaxillary and dentary teeth. In addition, some species of *Serrasalmus* and *Pristobrycon* have a tooth-bearing ectopterygoid (we do not discuss tooth replacement of the ectopterygoid here). In most pacus, both the upper jaw labial and lingual tooth rows are equally robust, whereas in the lower jaw, if lingual teeth are present, the number of teeth and the teeth themselves are smaller than the labial tooth row. Conversely, in *Tometes* and *Metynnis* the upper jaw labial row is reduced in size, and the lingual row is the primary upper dentition. Almost all pacus have a conspicuous overbite, with a thick lower lip comprised of adipose tissue which, when viewed externally, maintains a leading edge with the upper jaw (Figure 10). However, despite this apparent overbite, the labial dentary teeth are angled such that they generally occlude with the lingual premaxillary teeth. In pacus there are fewer, larger teeth in the lower jaw relative to

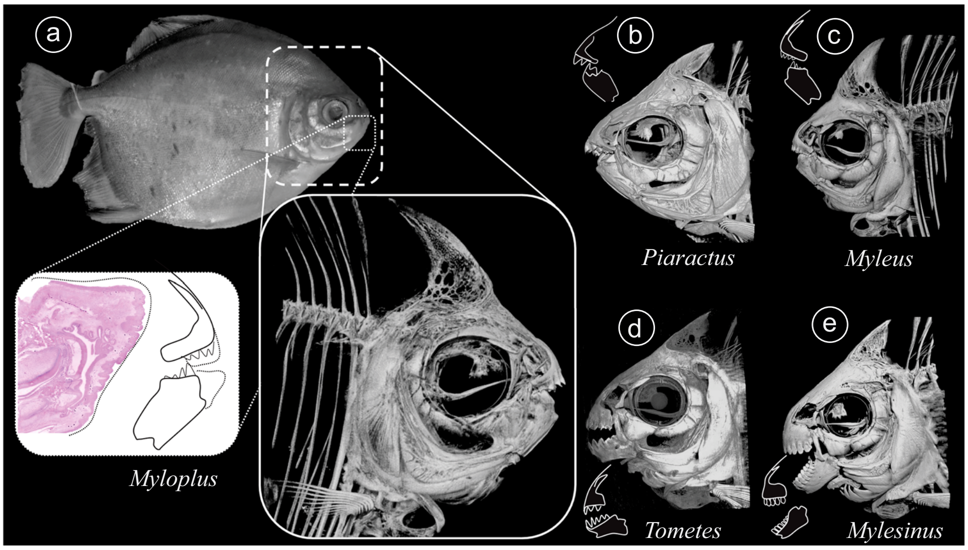


FIGURE 10 Differences in tooth morphology in pacus visualized with microcomputed tomography (CT) and histology. (a) *Myloplus torquatus* (FMNH 109794) specimen, with inset (left) showing histology of fatty lip that masks premaxillary prognathism and inset (right) showing CT of cranial skeleton. Skulls (b–e) show variation in pacu tooth and skull morphology with schematics outlining differences in pacus with overbites (a–c) and those without (d–e). (b) *Piaractus brachypomus* (ANSP 166685), (c) *Myleus setiger* (ANSP 197912), (d) *Tometes kranponhah* (ANSP 196745), (e) *Mylesinus paucisquamatus* (CAS 20221). Images not to scale [Color figure can be viewed at wileyonlinelibrary.com]

the upper jaw. Though there are similar numbers of teeth in the premaxillary labial and lingual tooth rows, in the dentary, there are always fewer lingual teeth than labial teeth.

Cusp shape varies considerably between genera and between species in pacus, but in general, upper jaw teeth have more rounded cusps whereas larger lower jaw teeth have triangular-shaped cusps. In medium-sized pacus like *Myloplus* and *Myleus*, the cusp angle among adjacent lingual and labial teeth alternates from contralateral to ipsilateral, making a "W"-shaped pattern when viewed ventrally across the dentition (e.g., see *Myloplus* in Figure 1d). In piranhas, teeth are either tricuspid (or pentacuspid in *Pygopristis denticulata*) with the smaller, lateral cusps used to interlock adjacent teeth together. More carnivorous piranhas (e.g., *Serrasalmus, Pygocentrus,* and some *Pristobrycon, P. calmoni*) have serrated, falciform teeth which interlock with adjacent teeth via lateral cusps, reminiscent of some sharks with an anterodorsal serrated edge and a posterior notch. Omnivorous piranhas have crenulate teeth with multiple short cusps for prying scales from prey (e.g., in *Catoprion* and juvenile *Pygopristis*) or tearing plant materials (in adult *Pygopristis*; Nico & Taphorn, 1988).

3.4 | Comparative methods: Evolution of replacement mode & heterodonty

We found unilateral tooth replacement in nearly all serrasalmids (Table 1). Exceptions occur in individuals with multiple developing rows of teeth in all jaws at once (bilateral replacement), albeit with one side usually more developed than the others. In cases where replacement pattern was difficult to diagnose, we generally noticed damage to the jaw. Unilateral tooth replacement is reconstructed as the ancestral state for all extant serrasalmids by both Bayesian and maximum likelihood-driven methods. Unilateral replacement does not correlate strongly with diet (Figure 10). *Serrasalmus manueli* showed indeterminate patterns of tooth replacement, this ambiguity stems partly from the single specimen available for this species in our data set. The only species where bilateral symmetry of tooth replacement was seen across multiple specimens was *Catoprion mento*, an obligate lepidophagous species (Figure 10). All pacus exhibited some degree of shape and size heterodonty, including monognathic heterodonty (in both jaws) and dignathic heterodonty. Only *Mylesinus paucisquamatus* did not exhibit size heterodonty in the upper jaw, although heterodonty is clear in the shape of the premaxillary labial and lingual tooth rows. Results for phylogenetic signal show a weak correlation between phylogeny and replacement ($K = 0.15$, $p = 0.87$).

4 | DISCUSSION

4.1 | Pacus and piranhas share the same tooth-replacement mode

Unilateral tooth replacement is not unique to piranhas, and is instead widespread across all serrasalmids, herbivores and carnivores alike, suggesting that this replacement pattern is exapted for hypercarnivory and arose first in plant-feeding ancestors. However, this pattern of unilateral replacement is not an "all or nothing" exercise, whereby only one side of the dentition is in the process of replacement, whereas the opposite side is not. Rather, replacement is asynchronous rather than sequential, with one side almost always being more developed than the other. Our histological findings show concurrent bilateral development of replacement teeth, which demonstrates the utility of combined radiographic and histological methods in studies of development, which allow us to visualize low mineral density tooth germs. We propose that unilateral replacement be considered a synapomorphy for Serrasalmidae, relative to their putative immediate outgroup, the hemiodontids (Arcila et al., 2017; Betancur-R et al., 2019) which have extraosseus replacement (Roberts, 1971, 1974). Serrasalmids provide another example of how intraosseus tooth replacement is often paired with polyphyodonty (continuous replacement), heterodonty, and large tooth size (Bemis & Bemis, 2015); the first documented example of this phenomenon in freshwater taxa. Intraosseus replacement of whole dental batteries, guided and supported by cancellous bone, helps to align developing teeth and guarantees structural integrity during intermediate phases of replacement.

4.2 | Constructional constraints on replacing an interlocking, heterodont dental battery

In serrasalmids, piecemeal replacement of individual teeth does not appear to be possible from a structural standpoint given their interlocking mechanisms. This serves as an extreme example of rigid constructional and developmental constraints acting on tooth development in a vertebrate. Almost all serrasalmids have some manner of interlocking teeth, although the diversity of these mechanisms makes their specific mechanical function (i.e., tooth-to-tooth loading regime) and homology difficult to assess (Figure 7). Furthermore, if the "peg & socket" interlocking mechanism in piranha teeth is homologous to the "clasp" mechanism in pacus, then this stands as another example of how dentitions in

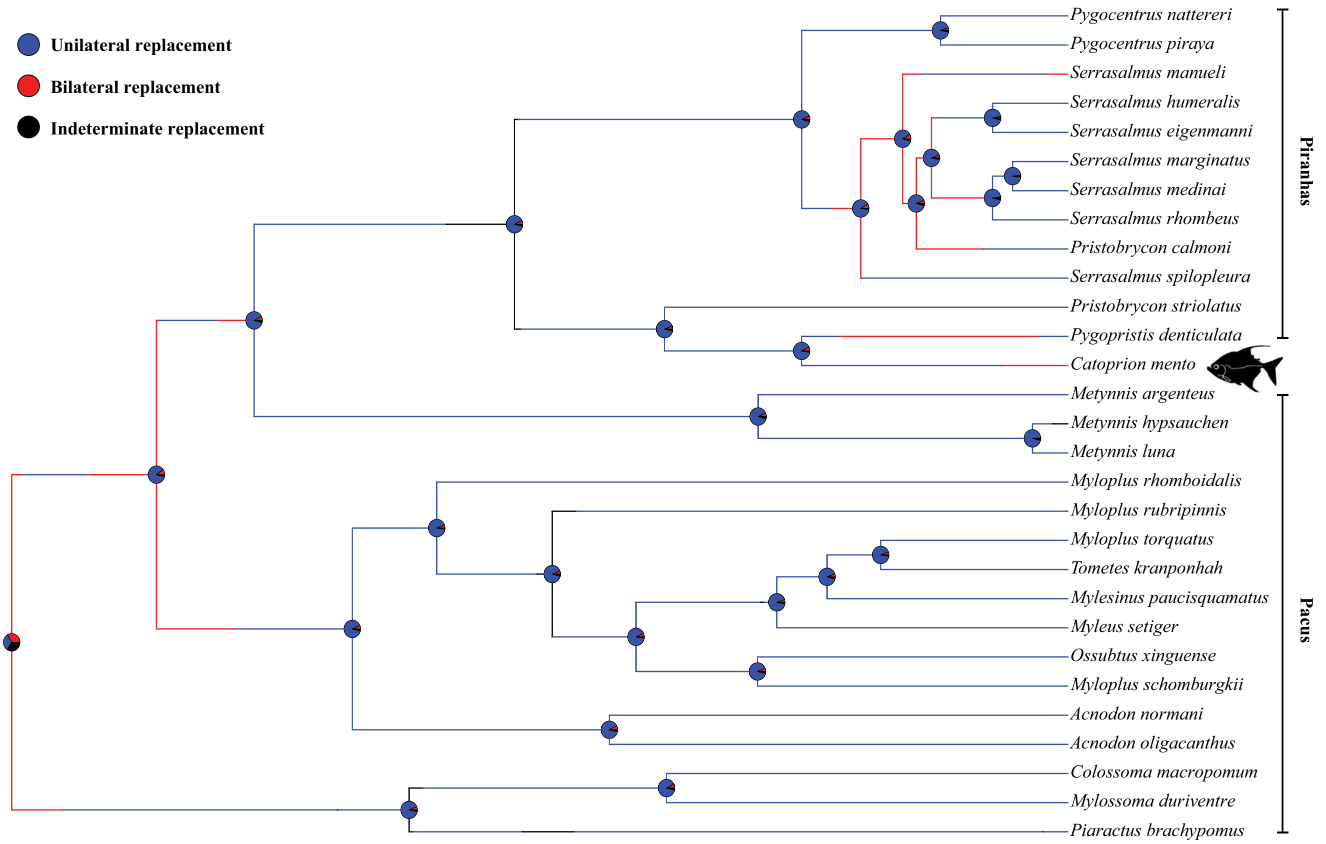


FIGURE 11 Evolution of tooth-replacement mode across the serrasalmids, using maximum likelihood (nodes) and stochastic character mapping (branches) methods. We could not determine replacement mode in *Metynnis hypsauchen*. Bilateral replacement patterns were found for *Serrasalmus manueli* and *Catoprion mento*. However, whereas our inference of replacement mode in *Serrasalmus manueli* is based on a single specimen, we found consistent (across multiple specimens) bilateral tooth replacement in *Catoprion mento*, an obligate lepidophagy. *Catoprion* is represented in the tree by an icon. Not represented: *Utiaritichthys* spp. Phylogeny from Thompson et al. (2014) [Color figure can be viewed at wileyonlinelibrary.com]

herbivorous serrasalmids are exapted for carnivory in piranhas. Serrasalmid teeth are also extremely large relative to other characins and appear to be tightly constrained in the number of teeth present, particularly in the upper jaw (Machado-Allison & Fink, 1996). The fact that teeth do not vary appreciably in number among piranha genera (exc. *Catoprion*; Gosline, 1951), relative to pacus (e.g., Myleinae), suggests stronger phylogenetic or constructional constraints on tooth size and number in piranhas (Figure 11).

The only taxon for which bilateral tooth replacement was found consistently among multiple individuals is the lepidophagous Wimple Piranha, *Catoprion mento*. *Catoprion* is also the only piranha that lacks an integrated tooth battery with interlocking teeth, and thus may not operate under the same adaptive constraints as other piranhas or other serrasalmids in general. Stout teeth in *Catoprion* must resist the impact with their host-prey and then subsequent leveraging of scales from prey (Janovetz, 2005). In contrast, interlocking teeth in other piranhas function to accentuate the saw-like nature of piranha dentitions during head shaking (Grubich, Huskey, Crofts, Orti, & Porto, 2012), which generate shear. Curiously, although many piranhas scale-feed at some point during their ontogeny, none appear to have the deintegrated tooth morphology *Catoprion* has. The bilateral tooth replacement of *Catoprion* is one of several ways that this species is specialized for obligate lepidophagy relative to other ectoparasitic piranhas (Kolmann, Huie, Evans, & Summers, 2018; Roberts, 1970; Sazima, 1983).

However, it may also be plausible that interlocking teeth arose after the evolution of unilateral replacement, at some point among stem serrasalmids. This is putatively supported by the complex diversity of teeth interlocking mechanisms, which are not ubiquitous across Serrasalmidae. Perhaps interlocking mechanisms became more exaggerated with great dental and diet diversity throughout serrasalmid history or perhaps that the tooth-replacement system evolved first, allowing for greater interlocking mechanisms to arise in turn.

4.3 | Why are interlocking teeth important for feeding in serrasalmids?

An evolutionary perspective on serrasalmid tooth replacement lends new insight into why interlocking dentitions are important to these fishes, because these mechanisms presumably evolved in an herbivorous, pacu-like ancestor, not a carnivorous piranha. Interlocking, simultaneously-replaced dentitions of herbivorous pacus provided consistently sharp teeth for grazing on cellulose- and silica-laden plant materials (Huie, Summers, & Kolmann, 2019; Norris & Prescott, 1959) and were well-situated to aid piranhas in gouging flesh from prey too. Trophic ecology in serrasalmids is less important for understanding tooth function than how teeth are engaging prey materials and how these materials are damaging teeth or inhibiting tooth function in turn. Curiously, the phenomenon whereby form (body shape, cranial morphology) and function (ecological niche) are decoupled seems widespread among characiform fishes (Burns & Sidlauskas, 2019; Huie et al., 2019). Selection should act on the orientation or shape of teeth that promote wearing in a way that avoids dysfunction (Ungar, 2015, Ungar & Williamson, 2000).

In contrast, the simultaneous replacement of teeth in serrasalmids requires a balance between rate of replacement and wear of the teeth. Although serrasalmids replace their teeth constantly, they do not do so often enough that teeth are exempt from wear or the microfractures that cause it. A tooth that pierces a prey item bears all the stress from a given load; within a contiguous dental battery, individual teeth benefit from neighboring teeth, which aid in dispersing local stresses. We hypothesize that the interlocking mechanisms found in serrasalmids mitigates localized stress distribution across the jaw by avoiding uneven wear across the battery of teeth. Stress results in fracturing (wear) and therefore a morphology that promotes equal stress dissipation also promotes equal wear (Evans & Sanson, 2003; Ungar, 2015). In other words, teeth that are locked together wear at similar rates, reducing the chance that overly worn individual teeth will degrade the function of the unit as a whole.

4.4 | Tales from the crypt: Do changes to the functional row (and crypt) precipitate tooth loss?

Our histological results show a great deal of cancellous bone and cartilage in the tooth crypts during development. How this heterogeneous skeletal material may affect jaw structural integrity is unknown. These same histological results also suggest that replacement is not driven solely by the developing dentition in the crypt. Rather, changes to the functional row and the underlying cancellous bone pedestal precipitate replacement (Figure 3; Bemis et al., 2005; Shellis & Berkovitz, 1976). As functional teeth are emptied of their inner, softer dental tissues, the underlying replacement teeth are directed towards these now-vacant pulp cavities (Morgan & King, 1983). We propose that the interior cavity of the functional row may serve as a guide for the underlying replacement dentition, allowing developing teeth to slip into their precise configurations with respect to flanking dentition (Bemis & Bemis, 2015; Shellis & Berkovitz, 1976). Cancellous bone surrounding the bony crypt is resorbed under the functional tooth row, allowing new teeth to rise into the vacant pulp cavity of older teeth.

These observations leave us with a “chicken or the egg” scenario: Is the functional row lost after a wear-induced threshold is reached (extrinsic or ecological control) or is tooth loss initiated by the eruption of the replacement teeth (and thereby, under intrinsic regulation; Fraser, Berkovitz, Graham, and Smith, 2006; Fraser, Graham, & Smith, 2006)? Our finding that replacement teeth may fit within the pulp cavity of functional teeth seems to suggest the latter: that the reduction in the cancellous bone of the jaw and the ascent of the replacement dental battery prompt shedding of the functional tooth row. This outcome suggests that replacement in piranhas only appears rapid because there is a very short latency period during which piranhas are equipped with a one-sided dentition (~5 days; Berkovitz & Shellis, 1978). This brief latency period is in stark contrast to the estimated “lifespan” of a given dental battery, reported as varying from 65 to 130 days (Berkovitz & Shellis, 1978) for *Serrasalmus rhombeus*. We suspect that given differences in the material and tribological (mechanical wear) properties of prey varying from seed husks to sinew and bone, that tooth wear and replacement rates will vary distinctively between piranhas and pacus, carnivores and herbivores.

ACKNOWLEDGMENTS

We thank G. Orti and H. Lopez-Fernandez for generously providing the tree used in our analyses. We thank the Hernandez lab for their aid and advice, especially J. Storch, M. Lefauve, and S. Keer. We especially thank the curators and collections staff at the Harvard Museum of Comparative Zoology (K. Hartel), Royal Ontario Museum (E. Holm and D. Stacey), Academy of Natural Sciences of Drexel University (M. Sabaj-Perez and M. Arce H.), California Academy of Sciences (D. Catania), Field Museum (C. McMahan), Burke Museum

(K. Maslenikov and L. Tornabene), Texas A&M collections (K. Conway and H. Prestridge), and the Auburn Museum (J. Armbruster and D. Werneke). Three anonymous reviewers provided excellent feedback and suggestions. We also thank R. Nagesan, H. Lopez-Fernandez, and R. Singer for providing CT scans of *S. altuvei* for figures. M. A. K. is supported by an NSF Postdoctoral Research Fellowship in Biology (1712015) and previously under a Friday Harbor Labs Postdoctoral Fellowship. K. E. B. was supported by NSF GRFP and VIMS Office of Academic Studies, Clyde D. & Lois W. Marlatt, Jr. Fellowship. F. J. I. was supported by NSF Dissertation Improvement Grant (BSR-85–14265) and NIH Predoctoral Fellowship (5-T32-GM07117).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

M. A. K., A. P. S., K. E. B., and K. E. C. developed the idea for the manuscript. K. E. C., L. P. H., and M. A. K. provided histological and light microscopy services, M. A. K., A. P. S., and K. E. C. supervised CT methods, F. J. I. and K. E. C. made scanning electron microscopy images. K. E. B. and F. J. I. provided critical edits and advice regarding tooth form, function, and development in the paper. All authors contributed to the writing of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kolmann MA, Cohen KE, Bemis KE, Summers AP, Irish FJ, Hernandez LP. Tooth and consequences: Heterodonty and dental replacement in piranhas and pacus (Serrasalmidae). *Evolution & Development*. 2019;e12306. https://doi.org/10.1111/ede.12306