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Body shape separates guilds of rheophilic herbivores (Myleinae: Serrasalmidae) better than feeding morphology

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ABSTRACT.—Herbivorous fishes feed on stems, leaves, flowers, seeds, fruits, and nuts of diverse aquatic plants, as well as algae. Pacus are the herbivorous cousins of piranhas and consume a myriad of diets comprised of these plant products, but a few species are phytophages, herbivores that feed almost exclusively on rapids-dwelling (rheophilic) riverweed plants from the family Podostemaceae. The degree to which pacus feed on riverweed varies from obligate year-round consumption to strictly seasonal, facultative feeding. Obligate phytophages feed heavily on riverweed and strictly occur in river rapids, while facultative phytophages only consume riverweed during seasons with low flow. Does ecological specialization (diet) beget morphological specialization in the feeding apparatus and/or body shape of phytophages? Under a phylogenetic framework, we used micro-computed tomography (µCT) scanning to compare functional feeding traits among 26 species of serrasalmids, four of which are obligate phytophages. We also compared body shape between pacus using geometric morphometrics to identify potential locomotor adaptations for rheophily. Obligate phytophages have dentitions and slicing jaws well-suited for shearing fleshy plant material relative to other pacus, which are equipped with fruit and seed crushing morphologies. Unrelated obligate phytophages have also converged on a similar body shape that is distinct from sympatric congeneric herbivores. Phytophagy involves more consistent changes to body shape than to feeding morphology, suggesting that body shape has more important ties to diet.

Keywords: geometric morphometrics, grazing, herbivores, rheophily, riverweed

INTRODUCTION

Piranhas and pacus (Serrasalmidae) are a conspicuous group of fishes that exploit a wide variety of dietary items in nearly every South American river basin. Piranhas are often considered as indiscriminate carnivores, although many species are more parasitic by nature, specializing on fin-feeding and scale-feeding (Nico, 1991; Sazima and Machado, 1990; Kolmann et al. 2018a). Unlike their piscivorous piranha cousins, pacus are primarily herbivorous and feed on a diverse range of terrestrial and aquatic plant material, as well as algae (Nico, 1991; Pouilly et al., 2004). That said, most species are seasonally or opportunistically omnivorous, and have extremely flexible diets that fluctuate across ontogeny, with food availability, water-levels, and habitat differences (Machado-Allison and Garcia, 1986; Nico, 1991; Correa and Winemiller, 2014). Pacu diets, in particular, are sensitive to the seasonal abundances and flowering phenology of their plant prey items (Boujard et al., 1990; Lucas, 2008; Correa and Winemiller, 2014; Correa et al., 2014). Among the myriad examples of serrasalmid trophic diversity are a few species of phytophagous pacus, herbivores that feed almost exclusively on constituents of the riverweed family Podostemaceae (Santos et al., 1997; Jégu et al., 2002; Andrade et al., 2016a, 2016b).

Species of Podostemaceae are aquatic vascular plants restricted to rapids (rheophily), a high-energy and challenging environment for fishes to live in (Philbrick and Retana, 1998) (Fig. 1). The plants utilize adhesive bacterial biofilms to adhere to rocky surfaces in waterfalls (Jäger-Zürn and Grubert, 2000), and undergo seasonal life history changes that correspond with changes in water flow. During wet seasons and times of high flow, Podostemaceae plants are entirely submerged. These plants lose their leaves

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and begin to flower after water levels recede during the dry season (Philbrick and Retana, 1998). Analyses on the stomach contents of phytophagous pacus have found that these fishes graze heavily on leaves during the wet season and flowers in the dry season (Jégu et al., 2003; Andrade et al., 2013, 2016b, 2017). Phytophagous pacus occur in the rapids near riverweed beds at all life stages, suggesting that riverweed provides a plentiful year-round food source throughout their ontogeny (Andrade et al., 2018). While riverweed remains an abundant resource, the swift flows of the rapids incur steep bioenergetic costs that make the plants largely inaccessible to most fishes, particularly small and poorly equipped juveniles.

Most pacus are somewhat rheophilic, but many species can only access rapids when water levels and flow permit. Therefore, most pacus also inhabit slow-moving environments (Boujard, 1990; Camargo et al., 2004; Zuluaga-Gómez et al., 2016). Similarly, the degree to which pacus feed on riverweed varies from obligate year-round consumption to strictly seasonal, facultative feeding. While obligate phytophages feed heavily on riverweed and occur only in rapids, facultative phytophages only consume riverweed during dry seasons with low flow (Boujard et al., 1990; Santos et al., 1997; Andrade et al., 2016a, 2016b). This begs the question of how dietary ecology and habitat are tied to changes in feeding morphology versus body shape in phytophagous serrasalmids. Have all phytophagous pacus converged on the same morphology or do they vary according to their degree of phytophagy? Do obligate phytophages exhibit jaw morphologies that enable them to consume riverweed more efficiently than other herbivores, and do their body shapes permit permanent residency in rapids?

Fig. 1. Patches of flowering riverweed (Podostomaceae) exposed on rocks in large rapids during the low water season in the Iriri River at cachoeira Grande, 3°50’35.5’’S, 52°44’08.3’’W, Brazil. A) 9 Sep 2013. B) 22 Sep 2015. Photos by M. Sabaj.

Our objectives were to (1) use micro-computed tomography (μCT) scanning and geometric morphometrics to compare the feeding morphology and body shape, respectively, of phytophagous pacus with other serrasalmids, (2) assess the link between phytophagy and morphology, and (3) characterize the morphological axes of variation in herbivorous pacus from the Lower Amazon in general.

MATERIAL AND METHODS

Specimen acquisition and ecological classification.— We examined 26 species of serrasalmids, with a least one representative from every genus in the family except *Utiaritichthys*. Specimens were acquired from museums or personal collections and were μCT scanned using the Bruker Skyscan 1173 at the Karel F. Liem Bio-Imaging Center at the University of Washington Friday Harbor Laboratories. Scans were conducted within a range of settings: 55-70 kV, 100-145 μA, and a voxel size of 25-57 μm. The reconstructed image stacks were converted to the DICOM file format and visualized using Horos (version 1.1.7; The Horos Project, 2015). A single individual per species was selected for this study. Because the size at maturity is uncertain for many species of serrasalmids, we attempted to select similar sized individuals (55.5–183.8 mm SL) with regards to the inherent size variability. All of the respective image stacks were made publicly available on MorphoSource.org. The MorphoSource media numbers and museum catalogue numbers are presented in Table 1.

In order to test the associations between diet and morphology, we conducted a literature review to classify the examined taxa into trophic groups. To account for the plasticity of serrasalmid diets, we took note of the most prominent prey items based on percent volume and occurrence as reported by diet studies across ontogeny and seasons (Table 1). Then we classified each species into one of six trophic categories: generalist herbivore (seeds, fruits, and other allochthonous plant material), lepidophage, omnivore, obligate phytophage, facultative phytophage, or piscivore. The piscivores included any

Table 1. Species examined and their respective specimen voucher, MorphoSource media number, diet, and habitat. Diet abbreviations: FP = facultative phytophage, H = generalist herbivore, L = lepidophage, OP = obligate phytophage, O = omnivore, and P = piscivore. Habitat abbreviations: FR = facultative rheophile, NR = non-rheophile, and OR = obligate rheophile. Alternative specimens used for geometric morphometrics are indicated with an asterisk (*). Museum codes are based on Sabaj (2019) except MK, which refers to the personal collection of Matthew A. Kolmann.

Species	**Museum & Catalog Number**	**MorphoSource Media Number**	**Diet**	**Habitat**	**Source**
Acnodon normani	CAS 20739	M15980	FP	FR	Andrade et al., 2016b
Acnodon oligacanthus	ROM 100851	M36550	FP	FR	Planquette et al., 1996
Catoprion mento	ROM 86225	M20357	L	NR	Nico, 1991
Colossoma macropomum	FMNH 78087	M36551	H	NR	Pouilly et al., 2004
Metynnis argenteus	AUM 22490	M36552	H	NR	Nico, 1991
Metynnis luna	CAS 11770	M16061	H	NR	Nico, 1991
Mylesinus paraschomburgkii	MK-18-004	-	OP	OR	Jégu et al., 1989
Mylesinus paucisquamatus	CAS 20221 (MK-18-002*)	M16129 -	H	OR	Vitorino Júnior et al., 2016
Myleus setiger	ANSP 197912	M16121	H	OR	Jégu and Santos, 2002
Myloplus schomburgkii	SU-CAS 70039	M16125	FP	FR	Nico, 1991
Myloplus rhomboidalis	SU-CAS 34504	M16040	FP	FR	Boujard et al., 1990
Myloplus rubripinnis	ANSP 199578	M16066	FP	FR	Correa and Winemiller, 2014
Myloplus torquatus	FMNH 109794	M16134	FP	FR	Correa and Winemiller, 2014
Mylossoma duriventre	SU-CAS 54683	M16070	H	NR	Pouilly et al., 2004
Ossubtus xinguense	ANSP 197392	M16123	OP	OR	Andrade et al., 2016b
Piaractus brachypomus	ANSP 166685	M15138	H	NR	Correa et al., 2014
Pristobrycon striolatus	ANSP 166906	M16116	O	NR	Machado-Allison and Garcia, 1986
Pygocentrus nattereri	CAS 71016 (FMNH 111306*)	M16060 (M16144*)	P	NR	Pouilly et al., 2004
Pygopristis denticulata	AUM 36164	M36533	H	NR	Nico, 1991
Serrasalmus eigenmanni	SU-CAS 21982	M16059	P	NR	Nico, 1991
Serrasalmus humeralis	FMNH 56969 (FMNH 56969*)	M36554 (M36555*)	P	NR	Nico, 1991
Serrasalmus manueli	ANSP 198551	M15141	P	NR	Nico, 1991
Serrasalmus rhombeus	FMNH 111315	M16143	P	FR	Pouilly et al., 2004
Serrasalmus spilopleura	FMNH 108506	M36556	P	NR	Pouilly et al., 2004
Tometes ancylorhynchus	MK-18-001	-	OP	OR	Andrade et al., 2016a
Tometes kranponhah	ANSP 196745	M16064	OP	OR	Andrade et al., 2016a

species that feed predominantly on fins or whole fishes. One species, *Pristobrycon striolatus*, exhibits equivalent herbivory and piscivory, and was thereby classified as an omnivore. We also used the literature review to determine the typical habitat of each species, and classified them as obligate rheophiles, facultative rheophiles (found in the rapids and floodplains), or non-rheophiles – those that only occur in slow moving waters (Table 1). For example, the eagle beak pacu, *Ossubtus xinguense*, is an obligate phytophage and obligate rheophile endemic to the Xingu River rapids in Brazil and occurs in sympatry with two other species treated as obligate phytophages/rheophiles, *Tometes ancylorhynchus* and *T. kranponhah*, the latter of which is also endemic to the Xingu (Andrade et al., 2016a; 2016b).

Inferring the co-evolution of phytophagy and rheophily.—We used a combined maximum likelihood and stochastic character mapping approach to infer the evolutionary history of phytophagy and rheophily via the [simmap] function in the *phytools* package in R (Huelsenbeck et al. 2003; Revell, 2012). For these SIMMAP analyses, we used a published, multi-locus, time-calibrated serrasalmid phylogeny (Thompson et al., 2014). Because *Tometes ancylorhynchus* and *Mylesinus paraschomburgkii* were not included in this phylogeny, we added them by replacing the tips of their closest relatives (*Myloplus planquettei* and *Tometes lebaili*, respectively) based on the relationships found in Machado et al. (2018). We also trimmed the phylogeny to include only recognized species for a total of 34 terminal taxa. Then we ran separate SIMMAP analyses on the discrete diet and habitat classifications with 1,000 iterations each using an 'equal-rates' model. The [drop.tip.simmap] function, also in the *phytools* package (Revell, 2012), was used to trim the phylogeny even further to include only the taxa examined in this study, while retaining the discrete character mappings on each branch. As a potential caveat, we note that the Thompson et al. (2014) phylogeny lacked a few species of known obligate phytophages and rheophiles, which may affect our results. However, this is the most comprehensive phylogeny published to date and still permits us to test general hypotheses surrounding the evolution of phytophagy and rheophily in serrasalmids.

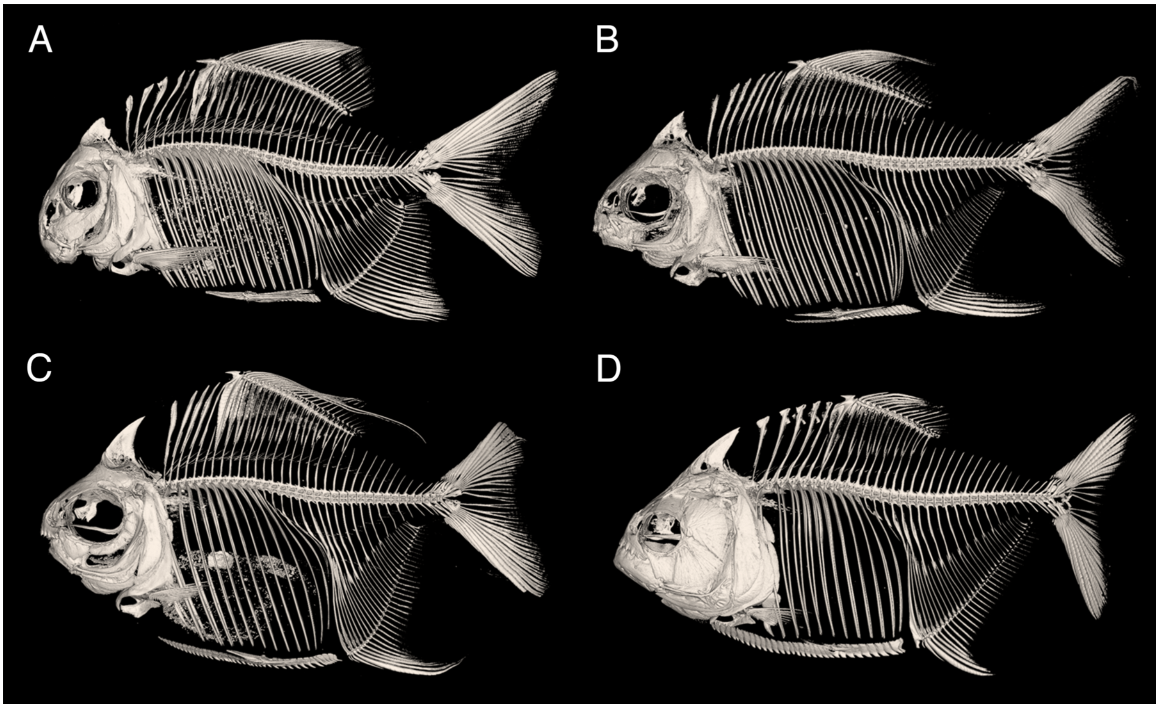
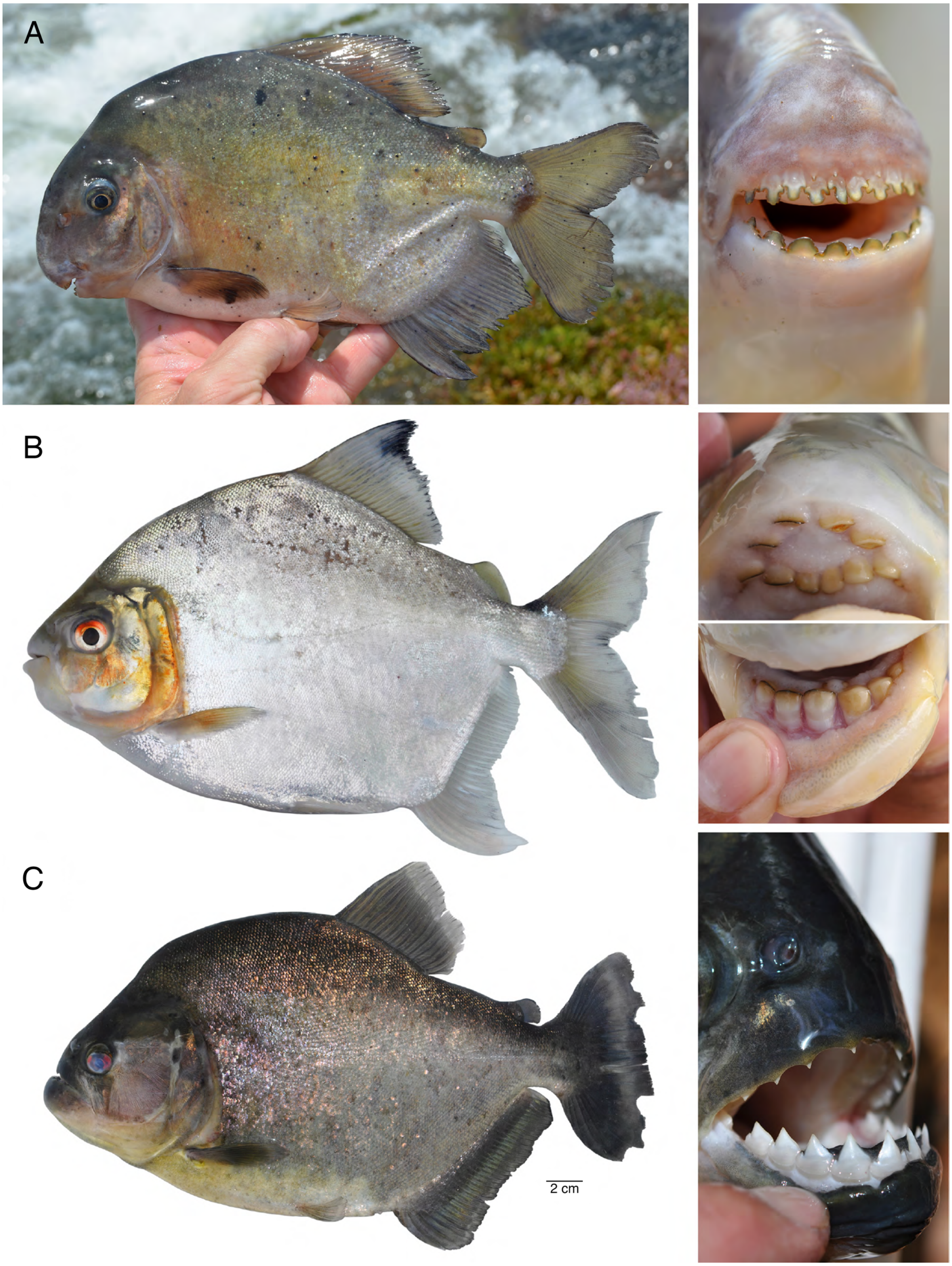


Fig. 2. (Above) Lateral µCT images of four serrasalmids that represent three trophic guilds: A) *Ossubtus xinguense* (phytophage), B) *Tometes kranponhah* (phytophage), C) *Myloplus schomburgkii* (herbivore), and D) *Serrasalmus spilopleura* (piscivore).

Fig. 3. (Page 5) Live photos and dentition of representatives from the three trophic guilds: A) Phytophagy (*Ossubtus xinguense*, not preserved), B) Herbivory (*Myloplus rhomboidalis*, ANSP 193059), C) Piscivory (*Serrasalmus rhombeus*, INPA 40315). Photos by M. Sabaj.


A
B
C
2 cm

Comparing feeding morphology between trophic guilds.—For clarity purposes, we used separate terminologies to distinguish between changes in the feeding apparatus versus body shape. Terms such as "feeding adaptations" and "feeding morphology" are strictly used to describe jaw morphology, even though a specialized body shape might be considered a feeding adaptation in this context.

To determine whether phytophagous pacus have a specialized feeding morphology, the µCT scans and Horos were used to measure several biomechanical predictors of feeding performance. We measured the following traits used by Kolmann et al. (2018a): (a) tooth aspect-ratio for assessing tooth shape (incisors vs. molariform teeth), (b) occlusional offset, an indicator of either slicing or crushing jaw action, (c) lower jaw length, (d) anterior and posterior mechanical advantage (AMA and PMA, respectively), a measure of jaw leverage and the trade-off between jaw closing speed and strength, and (e) the 2nd moment of area of the mandible, a proxy for jaw stiffness. The only changes made from Kolmann et al. (2018a) were the points along the jaw where we estimated 2nd moment of area. The first cross section was made at the anterior end of the mandible, just adjacent to the symphysis, and subsequent measurements were made along the long axis of the mandible at 25%, 50%, and 75% of its total length. The standard lengths of the preserved specimens were measured using digital images in ImageJ.

To account for the effects of size on our morphometric measurements, we conducted several phylogenetic linear regressions using the [phyl.resid] function from the *phytools* package (Revell, 2012). Each measurement was regressed against standard length except for tooth aspect ratio and mechanical advantage because they are dimensionless traits that are effectively already size-corrected. The phylogenetic residuals and raw values of the aforementioned traits were used as our size-corrected data. To reduce the dimensionality of the morphological variation and visualize the phylomorphospace occupied by each species, we used the [phyl.pca] function in *phytools* (Revell, 2012) to conduct a phylogenetically-explicit principal component analysis (phyPCA) on the size-corrected values using a correlation matrix (Sidlauskas, 2008).

To test for significant variation among the feeding traits between trophic groups, we performed phylogenetic MANOVA and ANOVA. In order to increase our statistical power, we reduced the number of diet categories from six to three (herbivory, phytophagy, piscivory; see Figs. 2 and 3), by synonymizing scale-feeding with piscivory as well as facultative phytophagy and omnivory with herbivory.

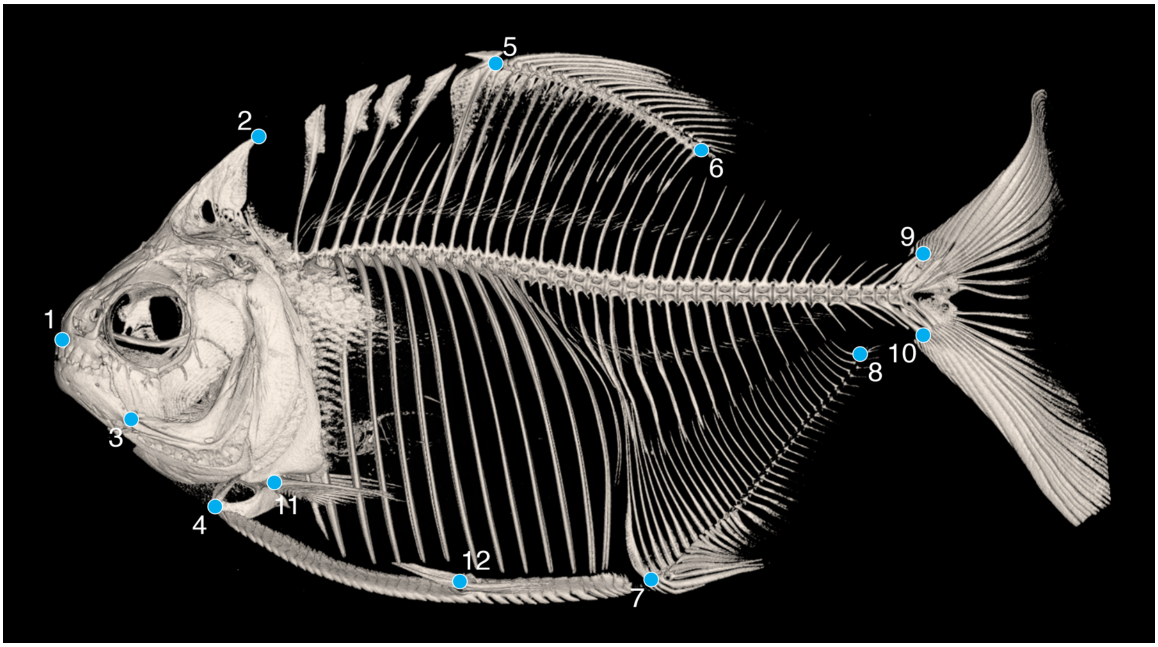


Fig. 4. Example specimen (*Pygopristis denticulata*) showing the 10 digital landmarks used for geometric morphometrics. 1) the tip of the snout, 2) the tip of the supraoccipital, 3) the jaw joint, 4) bottom of the pectoral girdle, 5) the most anterior part of the dorsal fin, 6) the most posterior part of the dorsal fin, 7) the anterior most part of the anal fin, 8) the posterior most part of the anal fin, 9) the top of the caudal peduncle, 10) the bottom of the caudal peduncle, 11) the pectoral fin joint, and 12) the pelvic fin joint.

To test the relationship between diet and jaw morphology, the [aov.phylo] function in the *geiger* package (Harmon et al., 2008) was used to perform a phylogenetic MANOVA (1,000 simulations and Wilks' λ) using the trophic classifications as the independent variable and the size-corrected data as the dependent variables. To determine which traits are influenced by diet, we ran a series of phylogenetic ANOVA and *post-hoc* pairwise tests (1,000 simulations and the Holm-Bonferroni method) on each size-corrected trait using the [phylANOVA] function in the *phytools* package (Revell, 2012).

Comparing body shape between trophic guilds.—To compare the body shape of the different trophic guilds, we used landmark-based geometric morphometrics to assess body shape variation. The volume rendering program, CTVox, was used to visualize our µCT scans and save left-facing lateral images of each selected specimen. In most cases, we used the same individual that we used to measure feeding traits, but for some species the quality of the specimen was not suited for geometric morphometrics and an alternative specimen was used instead (indicated in Table 1). We used the program *tpsDig2* (Rohlf, 2017) to digitally assign photos with twelve fixed landmarks that captured the skeletal outline of each fish (Fig. 4). The landmark data were used to perform a generalized Procrustes analysis with the [gpagen] function in the *geomorph* package in R (Adams et al., 2018). This type of analysis scales, rotates, and aligns the landmarks of each fish around the origin to maximize coordinate alignment and produce Procrustes coordinates that retain the shape of each specimen. To visualize the variation in a phylomorphospace, we conducted a non-phylogenetic principal component analysis on the Procrustes coordinates using the [plotTangentSpace] function in *geomorph* (Adams et al., 2018), and used the [phylomorphospace] function in *phytools* (Revell, 2012) to plot the PC scores with the phylogeny overlaid (Sidlauskas, 2008).

To determine whether phytophagous pacus have body shapes that are distinct from other trophic guilds, we conducted a phylogenetic Procrustes ANOVA and subsequent *post-hoc* pairwise tests using the [advanced.procD.lm] function in *geomorph* (Adams et al., 2018) with 1,000 iterations. The three diet categories were used as the independent variables. This analysis compares overall body shape variation but does not identify which portions of the body are the most variable between groups. To identify those traits, we performed additional phylogenetic ANOVAs and *post-hoc* pairwise tests on a subset of the cranial landmarks (# 1-4, and 11) and post-cranial landmarks (# 2, 4-12), respectively.

RESULTS

The SIMMAP analysis inferred that general herbivory was the ancestral feeding state for all serrasalmids (Fig. 5). Facultative phytophagy evolved from herbivory once in the ancestor of *Acnodon* + *Myloplus* + *Mylesinus* + *Myleus* + *Ossubtus* + *Tometes*, and then transitioned into obligate phytophagy in the ancestor of *Tometes* + [*Ossubtus* + *Myloplus schomburgkii*]. Facultative phytophagy was secondarily regained in a transition away from obligate phytophagy in *M. schomburgkii*, and lost in a reversion back to herbivory in the ancestor of the [*Myleus*] + [*Mylesinus* + *Myloplus torquatus*] clade. The SIMMAP inferred that obligate phytophagy most likely evolved a second time in *Mylesinus paraschomburgkii*. Meanwhile, omnivory evolved at the base of the piranha lineages ([*Pristobrycon* + [*Catoprion* + *Pygopristis*]] + [*Serrasalmus* + *Pygocentrus*]), and then lost in *Pygopristis* (herbivores). Piscivory evolved at the base of the *Pygocentrus* + *Serrasalmus* clade.

The second SIMMAP analysis inferred that living in slow moving waters was the ancestral state for all serrasalmids (Fig. 5). Facultative rheophily likely evolved around the same time facultative phytophagy did in the ancestor *Acnodon* + *Myloplus* + *Mylesinus* + *Myleus* + *Ossubtus* + *Tometes*. Some degree of rheophily also evolved separately in the piranha, *S. rhombeus*. Obligate rheophily likely evolved once in the ancestor of the [*Mylesinus* + *Myleus*] + [*Ossubtus* + *Tometes*] clade. *Myloplus schomburgkii* and *M. torquatus* are both nested within this clade but appear to have reverted back to facultative rheophily. We recognize that these results may be subject to change upon use of a more inclusive serrasalmid phylogeny.

The first four axes from the feeding trait phyPCA were retained visually via the Cattell scree test and accounted for 75.3% of the total morphological variation (Fig. 6). The loadings of the first four axes are presented in Table 2. PC1 explained 33.8% of the variation and was strongly associated with jaw occlusion, jaw length, and 2nd moment at 25%, 50%, and 75% of the total jaw length. PC2 explained 17.1% of the variance and was associated with posterior jaw leverage and 2nd moment near the symphysis. PC3 explained 13.7% of the variation and was associated with anterior jaw leverage. PC4 explained 10.7% of the variation and was associated with anterior jaw leverage and tooth shape.

The phylogenetic MANOVA showed that diet was associated with variation in the jaw morphology of the different trophic guilds (Wilks' $\lambda = 0.042$, $F = 6.843$, $p = 0.007$). Phylogenetic ANOVAs found that only posterior jaw leverage ($F = 26.423$, $p = 0.005$) and jaw length ($F = 20.351$, $p = 0.015$) were associated with diet. The piscivores had significantly greater posterior mechanical advantage

(PMA) than both the herbivores ($t = 6.664$, $p = 0.012$) and the phytophages ($t = 5.837$, $p = 0.014$). However, average PMA did not differ between herbivores and phytophages ($t = 1.081$, $p = 0.458$). The piscivores also had longer jaws than the phytophages ($t = 5.718$, $p = 0.024$), but not the other herbivores ($t = 5.300$, $p = 0.058$). The average herbivore also had a longer jaw than the phytophages, but the difference was not significant ($t = 2.057$, $p = 0.127$).

The phylogenetic ANOVA confirmed that the remaining traits did not differ by diet. The F-statistics and p-values for these traits were as follows: tooth shape (F = 3.278, $p = 0.404$), anterior mechanical advantage (AMA) (F = 6.508, $p = 0.207$), occlusional offset (F = 1.672, $p = 0.643$), 2nd moment of area near the symphysis (F = 3.127, $p = 0.415$), 2nd moment of area at 25% of the jaw length (F = 4.267, $p = 0.319$), 2nd moment of area at 50% of the jaw

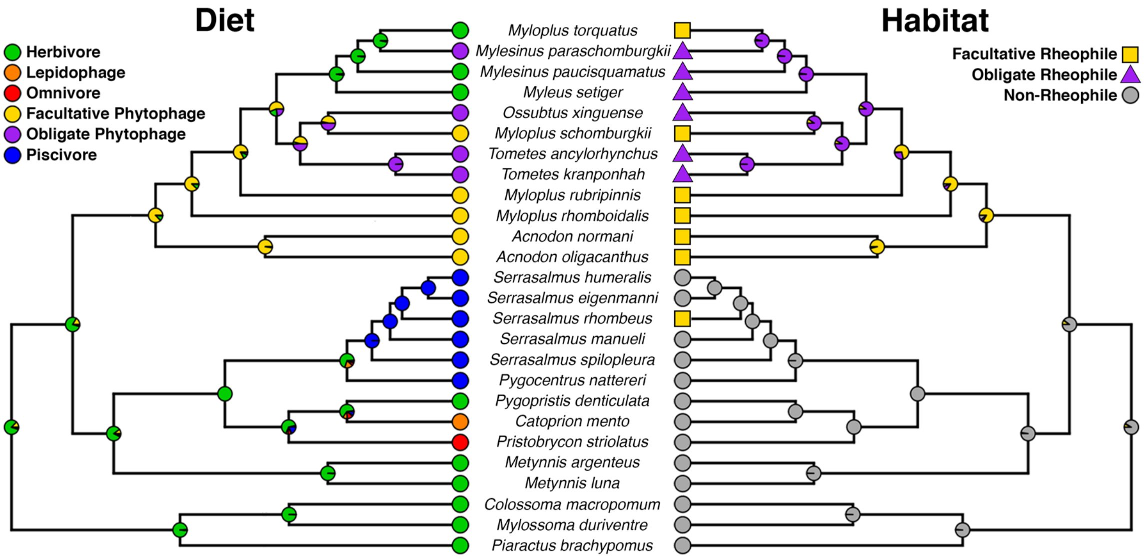


Fig. 5. Ancestral state reconstruction of diet (left) and habitat (right) in serrasalmids from two SIMMAP analyses performed on the Thompson et al. (2014) serrasalmid phylogeny with 34 terminal taxa. The trees shown here were later trimmed to show only the species examined this study. Note the similar evolutionary patterns between the different degrees of phytophagy and rheophily. On the left, the colors represent the different trophic guilds (green = herbivore, red = omnivore, orange = lepidophage, yellow = facultative phytophage, purple = obligate phytophage, and blue = piscivore). On the right, the colors and shapes represent different habitat associations (yellow squares = facultative rheophile, purple triangles = obligate rheophile, and grey circles = non-rheophile).

Table 2. Loadings of the first four principal component axes from the phyPCA performed on the size-corrected feeding traits.

Trait	PC1	PC2	PC3	PC4
Tooth Aspect Ratio	-0.373	-0.319	0.432	**-0.520**
Occlusional Offset	**0.747**	0.334	-0.145	-0.354
Jaw Length	**0.728**	0.027	0.502	0.001
Anterior Mechanical Advantage (AMA)	-0.029	-0.484	**-0.717**	-0.357
Posterior Mechanical Advantage (PMA)	-0.173	**-0.761**	0.013	**0.521**
2nd Moment of Area near Jaw Symphysis	0.400	**-0.657**	0.215	-0.318
2nd Moment of Area at 25% of Jaw Length	**0.646**	-0.026	-0.438	0.053
2nd Moment of Area at 50% of Jaw Length	**0.763**	-0.051	-0.033	0.242
2nd Moment of Area at 75% of Jaw Length	**0.792**	-0.271	0.136	0.072
% of variance	33.83	17.06	13.69	10.70
Eigenvalues	3.044	1.535	1.232	0.963

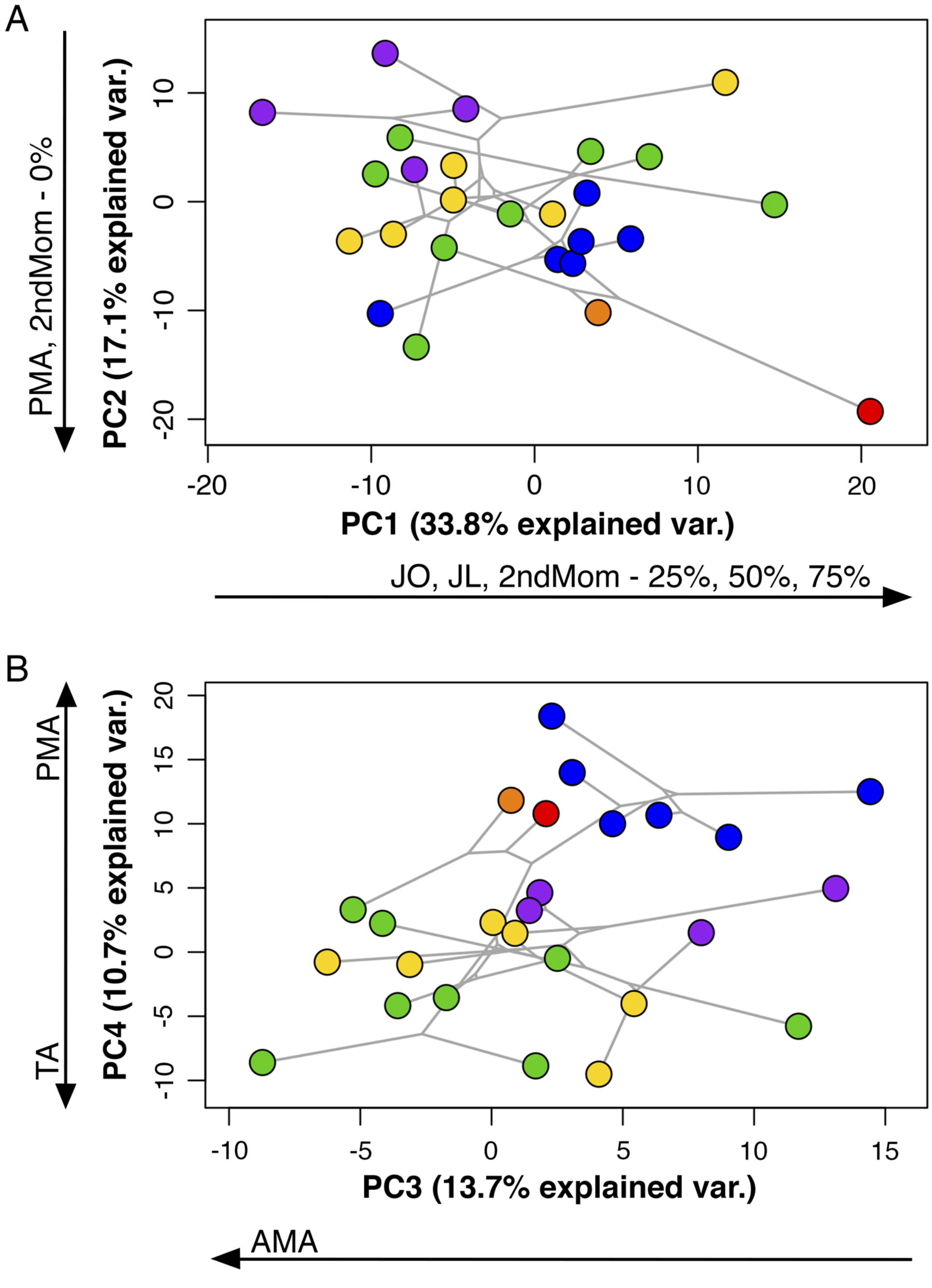


Fig. 6. Phylomorphospaces showing the size-corrected variation in serrasalmid feeding morphology. Colors represent the different trophic guilds (green = herbivore, red = omnivore, orange = lepidophage, blue = piscivore, purple = obligate phytophage, yellow = facultative phytophage). Abbreviations for the feeding traits are shown as TA (tooth aspect ratio), AMA (anterior mechanical advantage), PMA (posterior mechanical advantage), JL (jaw length), JO (jaw occlusion), and 2ndMom (2nd moment of area at 0%, 25%, 50%, or 75% of the total jaw length).

length (F = 1.165, *p* = 0.727), and 2nd moment of area at 75% of the jaw length (F = 1.249, *p* = 0.707). However, the pairwise tests comparing AMA between the different trophic guilds found significantly faster jaw closure (lower AMA) in phytophages compared to the herbivores (*t* = 3.599, *p* = 0.024). Even though the phytophages also exhibited lower AMA than the piscivores, no statistical difference was detected (*t* = 2.371, *p* = 0.584), nor was there a difference between the herbivores and piscivores (*t* = 1.718, *p* = 0.619).

The first three geometric morphometric PC axes were retained visually via the Cattell scree test and accounted for 82.2% of the total body shape variation (Fig. 7). PC1 explained 41.2% of the variation and was strongly associated with body depth, body elongation, anal-fin base length, and caudal peduncle length. PC2 explained 30.0% of the variation and was strongly associated with mouth orientation and dorsal-fin base length. PC3 explained 11.5% of the variation and was associated with head size and body elongation.

The results of the phylogenetic Procrustes ANOVA showed that there was no significant difference in the overall body shape among trophic guilds (F = 1.689, *p* = 0.09). However, a pairwise test did indicate a significant difference between the phytophages and herbivores (Z = 2.291, *p* = 0.025). No other pairwise comparisons found a significant difference between trophic guilds. The Procrustes ANOVA performed on the cranial landmarks found a significant difference in head shape between guilds (F = 2.170, *p* = 0.032). Phytophages differed from both the generalist herbivores (Z = 2.805, *p* = 0.007) and the piscivores (Z = 2.081, *p* = 0.033), but the herbivores and piscivores were not distinguishable from each other (Z = 0.809, *p* = 0.199). Meanwhile, the Procrustes ANOVA performed on the post-cranial landmarks also did not detect significant variation based on diet (F = 1.809, *p* = 0.076). However, the pairwise tests did indicate a significant difference between the phytophages and the herbivores (2.413, *p* = 0.024).

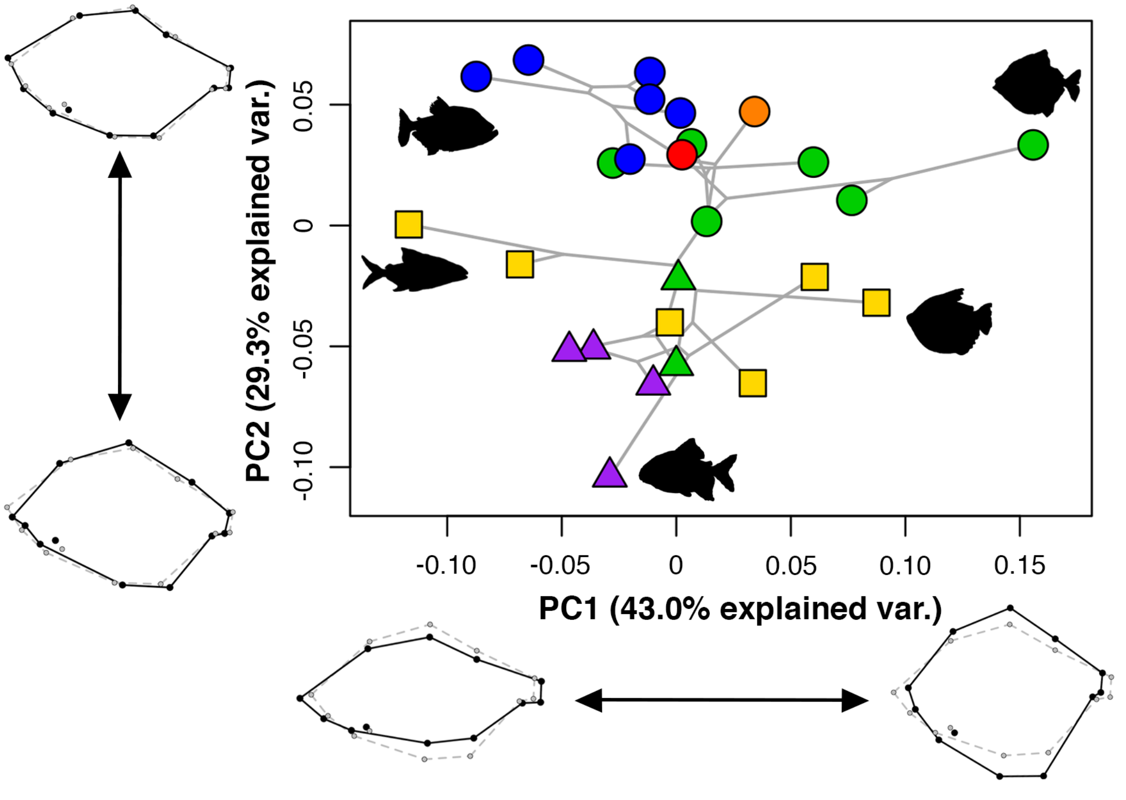


Fig. 7. Geometric morphometric phylomorphospace showing serrasalmid body shape variation. Colors and shapes represent the different trophic guilds and indicate rheophilic behavior, respectively. Green = herbivore, red = omnivore, orange = lepidophage, blue = piscivore, purple = obligate phytophage, yellow = facultative phytophage; circles = non-rheophilic, squares = facultative rheophile, and triangles = obligate rheophile. Black point plots show the major shape changes on each axis with respects to the consensus (gray outline).

DISCUSSION

Obligate phytophagy is a presumably narrow dietary niche that has evolved at least two times in the medium-sized pacus, yet we found few shared adaptations in their jaw morphologies. Our combined body and head landmark subsets show that an elongate, streamlined body plan with a narrow caudal peduncle, which favors swimming swiftly, is more tightly associated with eating riverweed than is cranial morphology. In other words, how your jaws work is less important than getting to where the food is. This is an example of the evolution of overall body shape obscuring morphological signals of dietary specialization in the skull. The facultative riverweed feeders (*Myloplus*, *Myleus*, *Acnodon,* etc.) can access riverweed material only twice a year, when river flow is low. The less streamlined bodies of these pacus are ill-suited to the demands of high-speed water – the year-round home of the obligate phytophages. This aligns well with the mismatch between jaw morphology and diet found in many broad studies of nearshore and reef fishes (Motta et al., 1995; Clifton & Motta, 1998; Claverie and Wainwright, 2004; Bellwood et al., 2005).

This is not to say that there is little variation in cranial morphology, but rather that skull variation does not correlate with archetypal specializations for obligate phytophagy. Though most pacus (*Myloplus, Myleus, Mylossoma,* etc.) have a typical characoid face, i.e. rounded with a terminal mouth, *Ossubtus*, *Tometes*, and *Mylesinus* have distinctive cranial forms. The first taxon has the only subterminal mouth found among serrasalmids, while the latter two have a forceps-like terminal 'beak' for browsing in the water column (Lujan and Conway, 2015; Andrade et al., 2016a, 2016b). Ecological observations support that *Ossubtus* scrapes plant matter from the substrate and therefore spends more time along the benthos relative to other phytophages (i.e., *Tometes, Mylesinus*; Andrade et al., 2018). Cranial morphology in *Ossubtus* resembles that of surgeonfishes, with broad incisiform teeth and quick jaw closure through low mechanical advantage (Purcell and Bellwood, 1993). This contrasts with the algae-grazing Lake Tanganyika cichlids (Tropheini tribe), with their high mechanical advantage jaws, or the scraping dentition of algae-feeding parrotfishes (Bellwood and Choat, 1990; Tada et al., 2017). Among these herbivorous lineages, the most apparent difference is that the pharyngeal jaws of cichlids and scarines are formidable vegetable processors, while the oral jaws of serrasalmids appear to do all of the work, as their pharyngeal jaws are gracile and poorly mineralized.

We also find that the degrees of phytophagy are closely associated with the degrees of rheophily (Fig. 5). We suspect that facultative rheophily first enabled opportunistic riverweed consumption by providing initial, albeit limited, access to the rapids. Whether the transition towards obligate rheophily evoked obligate phytophagy or *vice versa* remains uncertain. It seems more likely that changes in body shape facilitated obligate rheophily, which in turn provided the opportunity to specialize on riverweed. However, this does not provide a strong explanation for the minimal degree of phytophagy observed in two species of obligate rheophilic herbivores (*Myleus setiger* and *Mylesinus paucisquamatus*). Perhaps, these findings support the hypotheses that rheophiles are using alternative microhabitats in the rapids (i.e., those other than Podostemaceae beds; Andrade et al., 2018), or that obligate phytophagy is dependent on a symbiotic relationship with intestinal nematodes (Andrade et al., 2016b)

Browsers and grazers: examples from pacus.— Despite the poor fit between cranial morphology and diet in the riverweed specialists, some functional characteristics of the feeding apparatus in generalist pacus invite speculation about niche. Distinctions between pelagic and benthic phytophages (e.g., *Tometes* vs. *Ossubtus*, respectively), and even between these obligate phytophagous species and other pacus in general, recall the dichotomy between grazing and browsing in terrestrial ungulates. Grazers crop growing plant material at the substrate, while browsers eat mature plant matter above it. These different feeding strategies reflect differing foraging opportunities and resource variability. Browsers forage for diverse, yet patchy prey resources (e.g., shoots, fruits, seeds), while grazers feed consistently on a narrower range of ubiquitous foliage. Evolutionary transitions from browsing to grazing ecologies are frequently associated with changes from low- to high-crowned (brachyodont vs. hypsodont) dentitions, an adaptation for feeding on silicate-rich grasses and associated grit in drier climates (Simpson, 1951; Webb, 1977; Solounias & Semprebon, 2002).

We posit that in the context of the Xingu rapids, and South American cataracts writ large, obligate riverweed feeders are 'grazing' while facultative ones are 'browsing.' Riverweed is a grazable resource because it is ubiquitous along South American cataracts, but available year-round only to rheophilic taxa (e.g., phytophagous pacus). Phytophagous pacus have tightly packed rows of tall, multicuspid, incisiform teeth, while facultative feeders, for whom this is a temporally patchy resource, have at least one row of stout, molariform teeth (Figs. 8, 10). This distinction mirrors terrestrial herbivores wherein grazers have more blade-like teeth than browsers. The teeth of phytophagous *Ossubtus* are twice as tall as teeth from the more generalist herbivore *Myloplus rubripinnis*. Perhaps these differences in tooth shape make obligate phytophages better grazers, allowing for year-round foraging on riverweed foliage in arduous habitats (Franz-Odendaal & Solounias, 1984; but see Damuth & Janis, 2011). Another parallel between terrestrial herbivores and pacus stems from the arrangement and size of tooth cusps: obligate phytophages have tricuspid, spatulate teeth while other pacus have more robust, bicuspid teeth. A plurality of cusps is common for folivores versus their frugivorous relatives (Berthaume et al., 2013), as accessory cusps provide additional cutting edges, an adaptation for enhancing shear forces against prey (Fig. 9; Pouilly et al., 2004). These extra surfaces come at a price, however, as folivores and grazers incur higher costs in the form of damaging tooth wear (Berthaume et al., 2013). Nonetheless, the curious manner of tooth replacement in serrasalmids, in which entire dental batteries are shed and replaced as a unit, ensure that the dentition remains sharp and ready for feeding on prey materials (Shellis & Berkovitz, 1976; Kolmann et al., 2019). Pacus are like terrestrial herbivores, but unusual relative to other herbivorous fishes, in that they rely on the teeth in their oral jaws to both gather and process food. In contrast, many herbivorous fishes (e.g., parrotfish, tangs, and cichlids) use the teeth in their oral jaws for food acquisition and have a separate set of teeth in their pharyngeal jaws to process fibrous plant material (Tada et al., 2017). Consequently, most herbivorous fishes are harder to fit into a browser/grazer dichotomy.

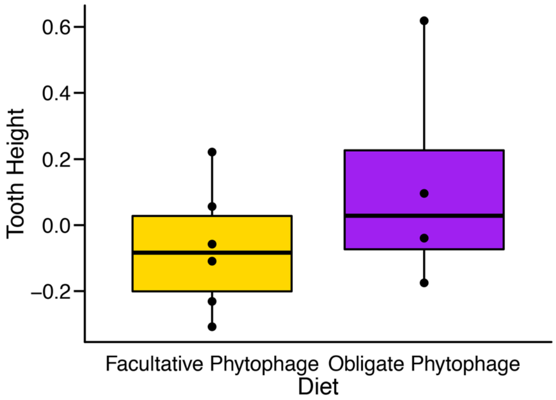


Fig. 8. Box plot comparing the average size-corrected tooth heights of facultative and obligate phytophages. Points represent a single observation or species. Average tooth height was not significantly different between the two trophic groups (F = 1.164, $p = 0.357$).

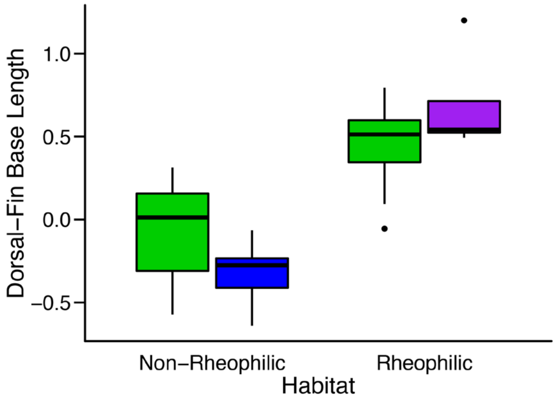


Fig. 9. Box plots showing average size-corrected dorsal fin base lengths for each trophic guild that occur within non-rheophilic and rheophilic environments (green = herbivore, blue = piscivore, and purple = phytophage). Points represent a single observation or species. Fin base length did not statistically differ between trophic guilds, but rheophilic pacus (facultative and obligate) had longer fin bases than non-rheophilic species (F = 38.512, $p = 0.032$).

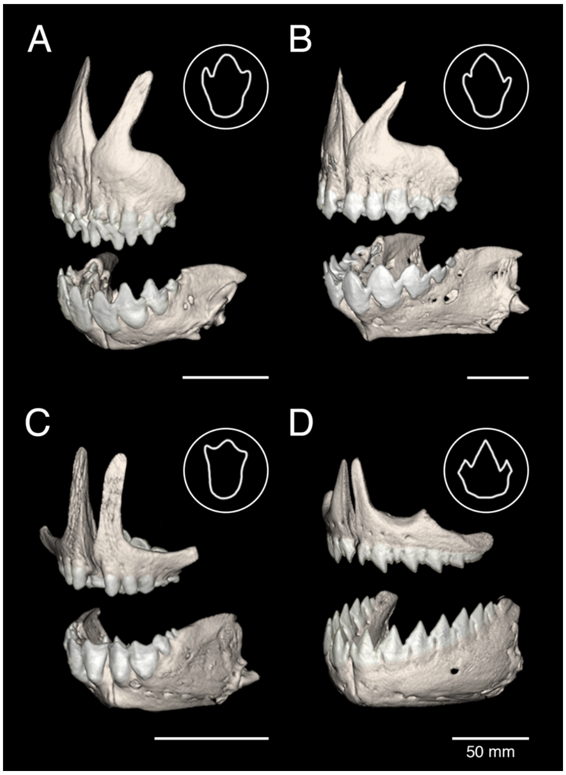


Fig. 10. A diagram of the pre-maxilla and mandible of four serrasalmids: A) *Ossubtus xinguense* (phytophage), B) *Tometes kranponhah* (phytophage), C) *Myloplus schomburgkii* (herbivore), and D) *Serrasalmus spilopleura* (piscivore). Depictions of the most anterior dentary tooth are also shown for each species (not to scale). Note that the phytophages have spatulate incisiform teeth for shearing riverweed, while the generalist herbivore has more robust dentition for crushing fruits and seeds.

Steady-swimming and rheophily: pacus can't be tunas.—Phytophagous pacus (and other rheophilic species) superficially share many of the same body-shape adaptations seen in steady swimmers (e.g., jacks and tunas), suggesting that life in high flow regimes goes hand-in-hand with steady, high speed, swimming (Webb, 1984; Langerhans, 2008). Marine pelagic species and rheophilic pacus converge on a stable-swimming design that minimizes drag through a stiff and streamlined body, coupled with a short caudal peduncle and high aspect ratio caudal fin to maximize thrust (Blake, 2004; Lujan and Conway, 2015). However, pacus that swim swiftly (and near constantly in rapids) have a longer-based dorsal fin rather than the subtle flow guides of tunas and mackerel (Webb, 1984). We propose the fundamentally unsteady nature of the flow in riverine environments leads to different stability requirements for rheophilic fishes. For phytophages, long-based medial fins may serve as significant propulsive structures to maintain a lateral position in river rapids. This aspect of swimming is not relevant to most pacus, thus explaining the shorter-based fins observed in non-rheophilic taxa (Jayne et al., 1996; Lauder and Druker, 2005; Fig. 9). An elongate dorsal-fin base, combined with larger pelvic fins (Kolmann et al., 2018b), provide lateral stabilization forces to counteract roll, as seen in other fishes with various body forms (Standen and Lauder 2005, 2007).

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