Supplementary Data for:

Histological phenotypes and complex prey processing behaviors in pacus

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Supplementary Methods:

We dissected museum specimens to observe lip, muscle, and tendon morphology, with particular attention paid to the primordial ligament and its association with the adductor mandibulae muscle series (adductor mandibulae division 1 = AM_1, adductor mandibulae division 2 = AM_2, etc.; Alexander, 1964; Datovo & Castro, 2012). The primordial ligament aids in adduction of the lower jaw in association with the adductor mandibulae complex (Alexander, 1964). Specimens were dissected on both sides, the infraorbital bones and opercular series removed to observe the underlying muscle tissue. Photographs were made with a handheld cellular camera phone for later reference and tracing.

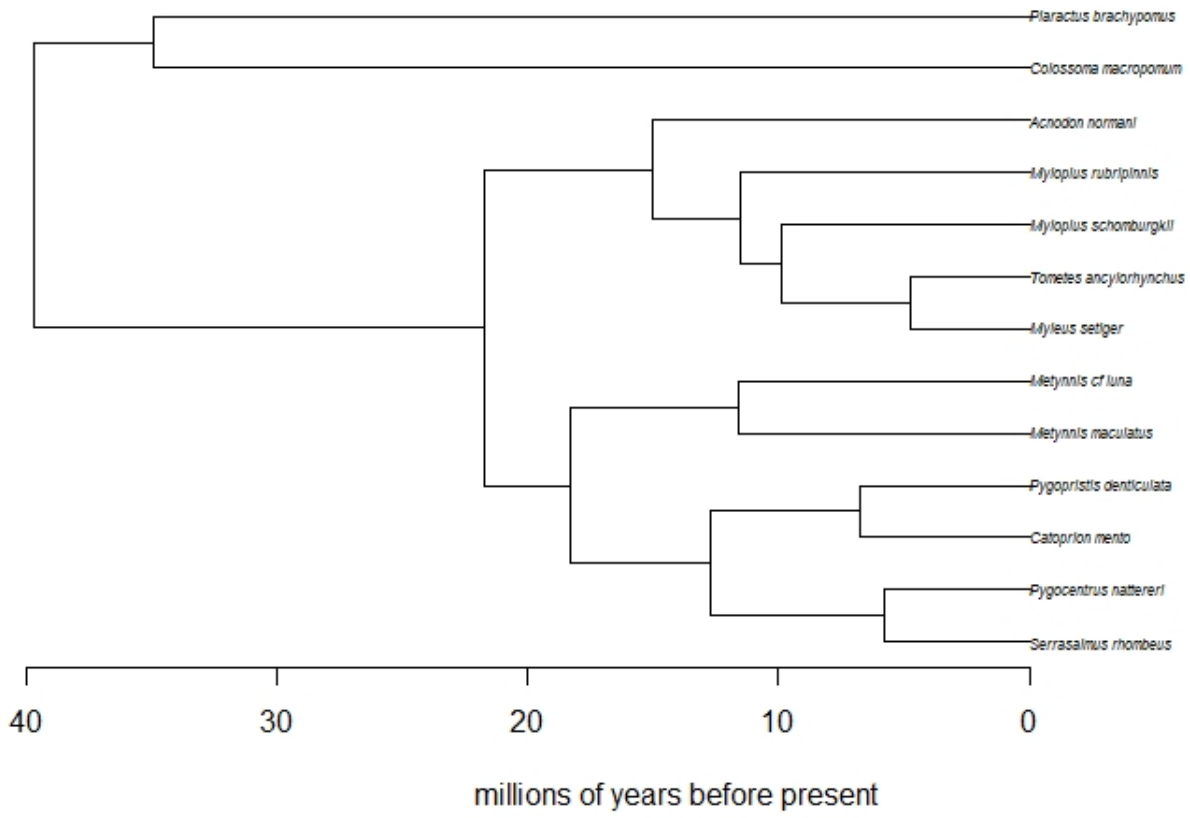
We also used contrast-enhanced micro-computed tomographic (microCT) imaging to visualize skeletal morphology in pacus and piranhas. Specimens were imaged using Friday Harbor Lab's Bruker 1173 Skyscan (Bruker Corp, Billerica, MA). Specimens were imaged at 65kV and 123uA with a 1.0 mm aluminum filter. In preparation for CT imaging, specimens were either wrapped in ethanol-moistened cheesecloth or slightly hydrated with 70% ethanol and heat-sealed within a plastic bag, then placed in a plastic tube, and stabilized with foam. Jaw anatomy was then visualized using volume rendering in the program 3DSlicer (www.slicer.org), following the Buser et al. (2020) CT segmentation and visualization workflow.

Appendix 1: Table of specimen sizes and histological methodology

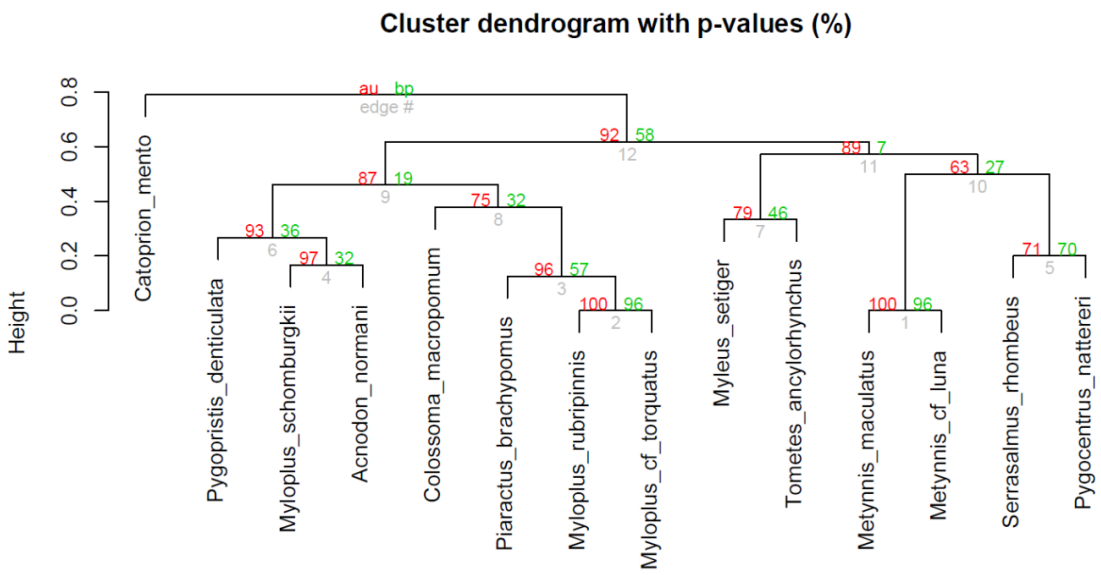
Specimen	Size (SL, mm)	Museum #	Section Thickness (um)	Stain
Piaractus brachypomus	37.8	FHL uncat.	3.5	Lee's Basic Fuchsin and Methylene Blue
Colossoma macropomum	51.0	UMMZ 252933	3.5	Lee's Basic Fuchsin and Methylene Blue
Acnodon normani	108.3	UMMZ 252922	3.0	Lee's Basic Fuchsin and Methylene Blue
Myloplus schomburgkii	47.2	UMMZ 252931	3.0	Lee's Basic Fuchsin and Methylene Blue
Tometes ancylorhynchus	91.0	UMMZ 252928	3.5	Lee's Basic Fuchsin and Methylene Blue
Myleus setiger	96.8	UMMZ 252926	3.5	Lee's Basic Fuchsin and Methylene Blue
Myloplus rubripinnis	86.7	UMMZ 252919	3.5	Lee's Basic Fuchsin and Methylene Blue
Myloplus cf. torquatus	67	UMMZ 252925	3.5	Lee's Basic Fuchsin and Methylene Blue

Metynnis maculatus	60	UMMZ 252930	3.0	Lee's Basic Fuchsin and Methylene Blue
Metynnis cf. luna	64.8	UMMZ 252927	3.5	Lee's Basic Fuchsin and Methylene Blue
Catoprion mento	30.0	ROM uncat.	3.0	Lee's Basic Fuchsin and Methylene Blue
Pygopristis denticulata	31.4	UMMZ 252932	3.5	Lee's Basic Fuchsin and Methylene Blue
Pygocentrus nattereri	32.0	UMMZ 252920	3.5	Lee's Basic Fuchsin and Methylene Blue
Serrasalmus rhombeus	37.3	ROM uncat.	3.5	Lee's Basic Fuchsin and Methylene Blue

Appendix 2: Trimmed phylogeny used for Comparative Methods (From Kolmann et al., 2020)



Appendix 3: Histological similarity clustering based on UPGMA estimation
(red values are approximately unbiased (AU) p-values and while green are bootstrap probability (BP) values, where AU is preferred)



Appendix 4: Diet Data References

Andrade, M. C., M. Jégu, and T. Giarrizzo. 2016a. *Tometes kranponhah* and *Tometes ancylorhynchus* (Characiformes: Serrasalmidae), two new phytophagous serrasalmids, and the first Tometes species described from the Brazilian Shield. Journal of Fish Biology 89(1): 467-94.

Andrade, M. C., D. B. Fitzgerald, K. O. Winemiller, P. S. Barbosa, and T. Giarrizzo. 2018. Trophic niche segregation among herbivorous serrasalmids from rapids of the lower Xingu River, Brazilian Amazon. Hydrobiologia: 1-16.

Andrade, M.C., Winemiller, K.O., Barbosa, P.S., Fortunati, A., Chelazzi, D., Cincinelli, A. and Giarrizzo, T., 2019. First account of plastic pollution impacting freshwater fishes in the Amazon: Ingestion of plastic debris by piranhas and other serrasalmids with diverse feeding habits. Environmental Pollution, 244, pp.766-773.

Dary, E.P., Ferreira, E., Zuanon, J. and Röpke, C.P., 2017. Diet and trophic structure of the fish assemblage in the mid-course of the Teles Pires River, Tapajós River basin, Brazil. Neotropical Ichthyology, 15(4).

González, N. & Vispo, C. 2002. Aspects of the diet and feeding ecologies of fish from nine floodplain lakes of the lower Caura, Venezuelan Guayana. Scientia Guaianae. 12: 329-3.

Goulding M. 1980. The fishes and the forest: explorations in Amazonian natural history. University of California Press, Berkeley.

Goulding M. Carvalho ML. Life history and management of the tambaqui (*Colossoma macropomum*, Characidae): an important Amazonian food fish. Revista Brasileira de Zoologia.1982;1: 107-133.

Honda EMS. Contribuicão ao conhecimento da biologia de peixesdo Amazonas: II. Alimentacão do tambaqui, *Colossoma bidens*. Acta Amazonica. 1974; 4: 47-53.

Kolmann, M.A., Hughes, L.C., Hernandez, L.P., Arcila, D., Betancur-R, R., Sabaj, M.H., López-Fernández, H. and Ortí, G., 2021. Phylogenomics of piranhas and pacus (Serrasalmidae) uncovers how dietary convergence and parallelism obfuscate traditional morphological taxonomy. Systematic Biology, 70(3), pp.576-592.

Leite, R.G. and Jégu, M., 1990. Régime alimentaire de deux espèces d'*Acnodon* (Characiformes, Serrasalmidae) et habitudes lepidophages de A. normani.

de Mérona, B., Dos Santos, G.M. and De Almeida, R.G., 2001. Short term effects of Tucuruí Dam (Amazonia, Brazil) on the trophic organization of fish communities. Environmental biology of fishes, 60(4), pp.375-392.

Lucas, C.M., 2008. Within flood season variation in fruit consumption and seed dispersal by two characin fishes of the Amazon. Biotropica, 40(5), pp.581-589.

Nico LG, Taphorn DC. Food habits of piranhas in the low llanos of Venezuela. Biotropica. 1988;20: 311-321.

Nico LG. 1991. Trophic ecology of piranhas (Characidae: Serrasalminae) from savanna and forest regions in the Orinoco River basin of Venezuela. Ph.D. dissertation, Univ. of Florida, Gainesville.

Pereira, TN, Castro, R. (2014). A new species of *Utiaritichthys* Miranda Ribeiro (Characiformes: Serrasalmidae) from the Serra dos Parecis, Tapajós drainage. Neotropical Ichthyology, 12, 397-402.

Ramos, I.P., Vidotto-Magnoni, A.P. and Carvalho, E.D., 2008. Influence of cage fish farming on the diet of dominant fish species of a Brazilian reservoir (Tietê River, High Paraná River basin). Acta Limnologica Brasiliensia, 20(3), pp.245-252.

Silva-Camacho, D.D.S., Santos, J.N.D.S., Gomes, R.D.S. and Araújo, F.G., 2014. Ecomorphological relationships among four Characiformes fish species in a tropical reservoir in South-eastern Brazil. Zoologia (Curitiba), 31(1), pp.28-34.

Vieira, I., and J. Gery. 1979. Differential growth and nutrition in *Catoprion mento* (Characoidei). Scale-eating fish of Amazonia. Acta Amazonica 9: 143-146.

Winemiller, K.O., 1989. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan ilanos. Environmental Biology of fishes, 26(3), pp.177-199.