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**Morphological Constraint in a Hyperdiverse
Lineage of Neotropical Tetras: A Geometric
Morphometric Analysis of Three Subfamilies of
Acestrorhamphidae (Teleostei: Characiformes)**

Mestrado

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“Make each day a new horizon. And when you want something in life, reach out and grab it. The core of man's spirit comes from new experiences. And there is no greater joy than to have an endlessly changing horizon, to see a different sun every day.”

- Christopher McCandless (em carta citada por Jon Krakauer, 1996)

Resumo

A ordem Characiformes representa um dos clados de peixes de água doce neotropicais mais diversos, morfológica e taxonomicamente. Dentro desse grupo, a família Acestrorhamphidae, recentemente delimitada, exibe alta riqueza de espécies. Porém, estudos prévios mostram que a morfologia de seus integrantes é altamente conservada, sendo um grande desafio para a sua sistemática e levanta questões sobre os processos evolutivos subjacentes a esse padrão. Diante disso, este estudo investiga a influência de fatores intrínsecos na evolução e cria um contexto filogenético temporalmente calibrado para análises de integração e modularidade de três subfamílias de Acestrorhamphidae: Pristellinae, Stethaprioninae e uma ainda não nomeada. Utilizando-se de técnicas de morfometria geométrica, buscou-se quantificar a variação e a covariação na morfologia do grupo sob dois principais objetivos: I- testar hipóteses de modularidade e integração morfológica com base em critérios funcionais e filogenéticos; II- avaliar como os padrões de integração e modularidade morfológica influenciam a evolubilidade e a disparidade morfológica em um grupo marcado por alta diversidade taxonômica, porém baixa diversidade morfológica aparente. A hipótese de três módulos (cranial, pós-cranial e caudal) apresentou a maior significância estatística. A modularidade apresentou sinal ligeiramente mais forte na ausência da filogenia, sugerindo que os padrões de covariação entre módulos refletem estruturas morfológicas intrínsecas não totalmente explicadas pela história evolutiva. Já nas análises de integração, as correlações foram ligeiramente mais altas com o uso da filogenia, indicando influência da herança comum. Destaca-se que o módulo caudal tende a apresentar maior independência, mesmo após considerar a filogenia. Notavelmente, a elevada integração morfológica observada sugere que o corpo evolui como uma unidade funcional coesa, limitando a variação independente entre regiões anatômicas. Essa forte coordenação entre partes do corpo atua como um fator intrínseco de restrição morfológica, canalizando a morfologia para trajetórias evolutivas específicas, neste caso relacionadas à hidrodinâmica e forrageamento, mas ela não impede a geração de diversidade, pelo contrário, pode até moldar a forma como essa diversidade emerge. Essas descobertas dialogam com estudos ecológicos e paleobiogeográficos para entender como a diversidade surge e se mantém.

Palavras-chaves: Diversificação; Morfologia; Evolução; Peixes Neotropicais; Morfometria Geométrica

Abstract

The order Characiformes represents one of the most morphologically and taxonomically diverse clades of Neotropical freshwater fishes. Within this group, the recently delimited family Acestrorhamphidae exhibits high species richness, yet previous studies have shown that its members display highly conserved morphology. This poses a significant challenge for ichthyological systematics and raises questions about the evolutionary processes underlying such a pattern. This study investigates the influence of intrinsic factors on morphological evolution and establishes a temporally calibrated phylogenetic framework for analyses of integration and modularity. A total of 32 species from three subfamilies: Pristellinae, Stethaprioninae, and one currently unnamed subfamily were analyzed. Using geometric morphometric techniques, we sought to quantify morphological variation and covariation within the group; test hypotheses of morphological modularity and integration based on functional and phylogenetic criteria; and assess how these patterns influence the evolvability and morphological disparity of a group characterized by high taxonomic diversity but low apparent morphological variation. We tested multiple modularity hypotheses under the null model of no modular structure, including a two-module configuration separating cranial and postcranial regions, and a three-module configuration comprising cranial, postcranial, and caudal modules. Notably, the caudal module consistently displayed greater independence than the cranial and postcranial modules, even after accounting for phylogeny. The high degree of morphological integration observed suggests that the body evolves as a cohesive functional unit, limiting independent variation between anatomical regions. This strong coordination among body parts acts as an intrinsic constraint on morphological change, channeling evolution along specific trajectories, particularly those related to hydrodynamics and foraging. However, it does not prevent the emergence of diversity; on the contrary, it may shape how such diversity arises. These findings complement ecological and paleobiogeographic studies seeking to understand how diversity originates and is maintained.

Keywords: Diversification; Morphology; Evolution; Neotropical Fish; Geometric Morphometrics

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1. Introduction

1.1. Diversification drivers

Why do some lineages explode into extraordinary diversity while others remain morphologically conservative over millions of years? What governs the tempo and mode of evolutionary change, and how do ecological opportunities, developmental architecture, and historical contingency interact to produce the patterns of biodiversity we observe today? These are central questions in macroevolutionary biology and answering them requires understanding not only *how* evolution proceeds, but also *why* it unfolds differently across clades and environments.

To that end, researchers have broadly categorized the drivers of diversification into two major classes: extrinsic and intrinsic factors. Extrinsic factors encompass environmental and ecological elements that influence species evolution externally, such as climate change, geological disturbances, ecological interactions (e.g., competition, predation), and niche availability (Clarcke & Halliday; Middleton, 2008; Goswami, 2016; Melo et al., 2022). These factors act upon the existing variation within a population, shaping the possible directions of natural selection.

Intrinsic factors, on the other hand, refer to internal characteristics of organisms that determine their capacity to vary and respond to selection. These include genetic features, morphological modularity and integration, phenotypic traits, and ontogenetic developmental processes (Goswami, 2006; Jablonski, 2022; Klingenberg, 2008; Olson & Miller, 1958). These factors structure phenotypic variation in a non-random manner, either promoting or constraining the directionality of evolutionary change.

Historically, macroevolutionary studies have emphasized extrinsic factors to explain major adaptive radiations, mass extinctions, and biodiversity patterns (Gould & Eldredge, 1977; Ricklefs, 2004). For instance, Melo et al. (2022) demonstrated that the rapid radiation of Characoidei during the Oligocene coincided with the emergence of novel habitats, highlighting the importance of ecological opportunity. At the same time, the persistence of older, morphologically conserved lineages points to the influence of historical stability and long-term accumulation of differentiation. Together, these observations suggest that both recent environmental change and deep-time processes have shaped Characiform diversity. Similarly, Dagosta and de Pinna (2019) stressed the importance of major geological events—such as the uplift of the Andes and reorganization of the Amazon Basin—in driving speciation. Additional studies have linked diversification in Loricariidae (Roxo et al., 2017), Cichlidae (López-Fernández et al., 2012), and other freshwater lineages to shifting water levels and altered

ADAMS,D. C.; ROHLF, F. J.; SLICE, D. E. Geometric morphometrics: ten years of progress following the 'revolution'. **Italian Journal of Zoology**, v. 71, n. 1, p. 5–16, 2004.

ADAMS,D. C. 2014. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. **Systematic Biology**. 63:685-697.

ADAMS,D. C. and M.L. Collyer. 2019. Comparing the strength of modular signal, and evaluating alternative modular hypotheses, using covariance ratio effect sizes with morphometric data. **Evolution**. 73:2352-2367.

ALBERT,J. S., & Reis, R. E. (Eds.). (2011). Historical Biogeography of Neotropical Freshwater Fishes. **University of California Press**.

ALBERT,J. S. ; Carvalho, Tiago P. (2011). Neogene assembly of modern faunas. In: Historical biogeography of neotropical freshwater fishes, pp. 119-136. **University of California Press**.

BETANCUR-R, Ricardo et al. Phylogenomic incongruence, hypothesis testing, and taxonomic sampling: The monophyly of characiform fishes. **Evolution**, v. 73, n. 2, p. 329-345, 2019.

BLACK,C. R., & Armbruster, J. W. (2022). Evolutionary Integration and Modularity in the Diversity of Suckermouth Armored Catfishes. **Royal Society Open Science**, 9(11), 220713.

BLOMBERG S. P. ,Garland T., Ives A.R.. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. **Evolution**, 57:717-745.

BOOKSTEIN,F. L. Principal warps: thin-plate splines and the decomposition of deformations. **IEEE Transactions on Pattern Analysis and Machine Intelligence**, v. 11, n. 6, p. 567–585, 1989.

BOOKSTEIN,F. L. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge: **Cambridge University Press**, 1991.

BOOKSTEIN,F. L. Combining the tools of geometric morphometrics. **Advances in morphometrics**, p. 131-151, 1996.

BORGES, P. P. et al. Stream fish metacommunity organisation across a Neotropical ecoregion: The role of environment, anthropogenic impact and dispersal-based processes. **PLoS One**, v. 15, n. 5, p. e0233733, 2020.

BOUCKAERT,R. R. et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. **PLoS Computational Biology**, v. 15, n. 4, e1006650, 2019. DOI: 10.1371/journal.pcbi.1006650.

BOUCKAERT,R. R. An efficient coalescent epoch model for Bayesian phylogenetic inference. **Systematic Biology**, syac015, 2022. DOI: 10.1093/sysbio/syac015.

BOUCKAERT,R. FixedTreeAnalysis – Tree Set Tutorial. **GitHub**, 9 jun. 2022. Disponível em: <https://github.com/rbouckaert/FixedTreeAnalysis/blob/master/doc/tree-set-tutorial/README.md>. Acesso em: 25 mar. 2025.

BOULENGER,G. A. (1895). Account of a large collection of fishes formed by Dr. C. Ternetz at various localities in Matto Grosso and Paraguay. **Proceedings of the Zoological Society of London**, 1895(3), 523-529.

BUCKUP,P. A. (1998). Relationships of the Characidiinae and phylogeny of characiform fishes (Teleostei: Ostariophysi). In: Malabarba, L. R., Reis, R. E., Vari, R. P., Lucena, Z. M. S., & Lucena, C. A. S. (Eds.), Phylogeny and classification of neotropical fishes. **EDIPUCRS**, Porto Alegre, Brazil, pp. 123–144.

BURNS, M. D. ; COLLYER, M. L.; SIDLAUSKAS, B. L. Simultaneous integration and modularity underlie the exceptional body shape diversification of characiform fishes. **Evolution**, v. 77, n. 3, p. 746-762, 2023.

BURNS, Michael D.; SIDLAUSKAS, Brian L. Ancient and contingent body shape diversification in a hyperdiverse continental fish radiation. **Evolution**, v. 73, n. 3, p. 569-587, 2019.

BÜHRNHEIM,C. M. et al. A new genus and species of characid fish from the Amazon basin: the recognition of a

relictual lineage of characid fishes. **Zootaxa**, n. 1886, p. 54–66, 2008. DOI: 10.11646/zootaxa.1886.1.4.

CHANG, J., Foresti, F., Alfaro, M. E., & Oliveira, C. (2019). Phylogenomic reappraisal of the Neotropical catfish family Loricariidae (Teleostei: Siluriformes) using ultraconserved elements. **Molecular Phylogenetics and Evolution**, 135, 148–165. <https://doi.org/10.1016/j.ympev.2019.02.017>

CLAVERIE, T., & Patek, S. N. (2013). Modularity and rates of evolutionary change in a power-amplified prey capture system. **Evolution**, 67(11), 3191–3207.

COLLYER, M. L., E.K. Baken, & D.C. Adams. 2022. A standardized effect size for evaluating and comparing the strength of phylogenetic signal. **Methods in Ecology and Evolution**. 13:367–382.

CONITH, A. J., & Albertson, R. C. (2021). The cichlid oral and pharyngeal jaws are evolutionarily and genetically coupled. **Nature Communications**, 12(1), 5477.

DAGOSTA, F. C. P., & Pinna, M. (2019). The fishes of the Amazon: Distribution and biogeographical patterns. **Neotropical Ichthyology**, 17(3), e190033.

DOUGLAS, J.; ZHANG, R.; BOUCKAERT, R. Adaptive dating and fast proposals: Revisiting the phylogenetic relaxed clock model. **PLoS Computational Biology**, v. 17, n. 2, e1008322, 2021. DOI: 10.1371/journal.pcbi.1008322.

DRUMMOND, A. J. et al. Relaxed phylogenetics and dating with confidence. **PLoS Biology**, v. 4, n. 5, e88, 2006. DOI: 10.1371/journal.pbio.0040088.

DRYDEN, I. L., & Mardia, K. V. (1998). Statistical Shape Analysis. **Wiley**, Chichester, England.

DRYDEN, Ian L.; MARDIA, Kanti V. Statistical shape analysis: with applications in R. **John Wiley & Sons**, 2016.

DU, Trina Y., Tissandier, Sylvie C., & Larsson, Hans C. E. (2019). Integration and modularity of teleostean pectoral fin shape and its role in the diversification of acanthomorph fishes. **Evolution**, 73(2), 401–411.

DUDGEON, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. **Biological Reviews**, 81(2), 163–182.

ELSHERBINI, J. et al. Dynamic Adaptive Evolution and Evolutionary Modularity and Integration in Catfishes of the Superfamily Doradoidea. **Ichthyology & Herpetology**, v. 112, n. 4, p. 552–566, 2024.

ESCHMEYER, W., Fricke, R., & Van der Laan, R. (2016). Catalog of Fishes: Genera, Species, References. **California Academy of Sciences**.

EVANS, K. M., Vidal-García, M., & Tagliacollo, V. A. (2019). Bony Patchwork: Mosaic Patterns of Evolution in the Skull of Electric Fishes (Apteronotidae: Gymnotiformes). **Integrative and Comparative Biology**, 59(2), 420–431.

FAIRCLOTH, B. C. (2016). PHYLUCES is a software package for the analysis of conserved genomic loci. **Bioinformatics**, 32(5), 786–788. <https://doi.org/10.1093/bioinformatics/btv646>

FELICE, R. N., Randau, M., & Goswami, A. (2018). A fly in a tube: Macroevolutionary expectations for integrated phenotypes. **Evolution**, 72(12), 2580–2594.

FINK, W. L., & Weitzman, S. H. (1974). The so-called "Cheirodontin" fishes of Central America with comments on the phylogeny of New World characiforms. **Smithsonian Contributions to Zoology**, 172, 1–46.

FIŠER, Cene; KOSELJ, Klemen. Coexisting cryptic species as a model system in integrative taxonomy. In: MONRO, Alexandre K.; MAYO, Simon J. (Org.). Cryptic species: morphological stasis, circumscription, and hidden diversity. **Cambridge: Cambridge University Press**, 2022. p. 169–196. (Systematics Association Special Volume Series, n. 89)

FLAMMANG, B. E.; LAUDER, G. V. Caudal fin shape modulation and control during acceleration, braking and backing maneuvers in bluegill sunfish, *Lepomis macrochirus*. *Journal of Experimental Biology*, v. 212, n. 2, p. 277-286, 2009.

GALTON, Francis. Regression towards mediocrity in hereditary stature. **The Journal of the Anthropological Institute of Great Britain and Ireland**, v. 15, p. 246-263, 1886.

GAYON, Jean. History of the concept of allometry. **American zoologist**, v. 40, n. 5, p. 748-758, 2000.

GERNHARD, T. The conditioned reconstructed process. **Journal of Theoretical Biology**, v. 253, n. 4, p. 769–778, 2008. DOI: 10.1016/j.jtbi.2008.04.005.

GERSTNER, C. L. Maneuverability of four species of coral-reef fish that differ in body and pectoral-fin morphology. **Canadian Journal of Zoology**, v. 77, n. 7, p. 1102–1110, 1999.

GIBSON, R. N. Migration and homing in intertidal fishes. In: CHEAL, A. J. et al. (Eds.) **Behaviour of Teleost Fishes**. London: Chapman and Hall, 1988. p. 97–109.

GILL, T. N. (1858). Synopsis of the fresh water fishes of the western portion of the island of Trinidad, W. I. **Annals of the Lyceum of Natural History of New York**, 6(10-13), 363-430.

GOMES, L. C., Pessali, T. C., Sales, N. G., Pompeu, P. S., & Carvalho, D. C. (2015). Integrative taxonomy detects cryptic and overlooked fish species in a neotropical river basin. **Genetica**, 143, 581-588. <https://doi.org/10.1007/s10709-015-9856-z>

GOODALL, C. R. (1991). Procrustes methods in the statistical analysis of shape. **Journal of the Royal Statistical Society: Series B (Methodological)**, 53, 285-339.

GOSWAMI, A. , & Polly, P. D. (2010). Methods for studying morphological integration and modularity. **Paleontological Society Papers**, 16, 213-243.

GOULD, S. J., & Eldredge, N. (1977). Punctuated equilibria: The tempo and mode of evolution reconsidered. **Paleobiology**, 3(2), 115-151.

GOULD, S. J., & Lewontin, R. C. (1979). The spandrels of San Marco and the Panglossian paradigm: A critique of the adaptationist programme. **Proceedings of the Royal Society of London. Series B. Biological Sciences**, 205(1161), 581-598.

GOWER, J. C. Generalized procrustes analysis. **Psychometrika**, v. 40, n. 1, p. 33-51, 1975.

HARMON, L. J. et al. Early bursts of body size and shape evolution are rare in comparative data. **Evolution**, v. 64, n. 8, p. 2385-2396, 2010.

HALLIDAY, T. J. D.; Goswami, A. Eutherian morphological disparity across the end-Cretaceous mass extinction. **Biological Journal of the Linnean Society**, v. 118, n. 1, p. 152-168, 2016

HENNIG, Willi. Phylogenetic systematics. **University of Illinois Press**, 1999.

HO, S. Y. W.; Phillips, M. J. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. **Systematic Biology**, v. 58, p. 367–380, 2009.

HUGHES, L. C., Orti, G., Weissensteiner, M., et al. (2021). Comprehensive phylogeny of catfishes (Siluriformes) using ultraconserved elements. **Systematic Biology**, 70(1), 91-105. <https://doi.org/10.1093/sysbio/syaa040>

HUXLEY, J. (1942). Evolution: The Modern Synthesis. **Allen & Unwin**.

HUXLEY, J. S. Constant differential growth-ratios and their significance. **Nature**, v. 114, n. 2877, p. 895-896, 1924.

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE. International Code of Zoological

Nomenclature. 4. ed. London: **International Trust for Zoological Nomenclature**, 1999.

JABLONSKI, David. Evolvability and macroevolution: overview and synthesis. *Evolutionary Biology*, v. 49, n. 3, p. 265-291, 2022.

JARVIS, E. D., et al. (2014). Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science*, 346(6215), 1320-1331.

JOLLIFFE, I. T. (2002). Principal Component Analysis (2nd ed.). **Springer**, New York, NY, USA.

KATOH, K.; STANDLEY, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution*, v. 30, n. 4, p. 772-780, 2013.

KENDALL, David G. Shape manifolds, procrustean metrics, and complex projective spaces. *Bulletin of the London mathematical society*, v. 16, n. 2, p. 81-121, 1984.

KALYAANAMOORTHY, S. et al. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, v. 14, p. 587–589, 2017. DOI: .

KLINGENBERG, C. P. Evolution and development of shape: integrating quantitative approaches. *Nature Reviews Genetics*, v. 11, p. 623–635, 2010.

KLINGENBERG, Christian Peter. Size, shape, and form: concepts of allometry in geometric morphometrics. *Development genes and evolution*, v. 226, n. 3, p. 113-137, 2016.

KLINGENBERG, Christian Peter. Morphological integration and developmental modularity. *Annual review of ecology, evolution, and systematics*, v. 39, n. 1, p. 115-132, 2008.

KLINGENBERG, C P. Morphometrics and the role of the phenotype in studies of the evolution of developmental mechanisms. *Gene*, v. 287, n. 1-2, p. 3-10, 2002.

KLINGENBERG, Christian Peter; MARUGÁN-LOBÓN, Jesús. Evolutionary covariation in geometric morphometric data: analyzing integration, modularity, and allometry in a phylogenetic context. *Systematic biology*, v. 62, n. 4, p. 591-610, 2013.

KLINGENBERG, Christian Peter. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biological Reviews*, v. 73, n. 1, p. 79-123, 1998.

LAROUCHE, O. ; ZELDITCH, M. L.; CLOUTIER, R. Modularity promotes morphological divergence in ray-finned fishes. *Scientific Reports*, [S.l.], v. 8, n. 7278, p. 1–10, 2018. DOI: 10.1038/s41598-018-25715-y.

LEAL-CARDÍN, Mariana et al. Signatures of convergence in Neotropical cichlid fish. *Molecular Ecology*, v. 33, n. 19, p. e17524, 2024.

LIMA, Flávio C. T.; WOSIACKI, Wolmar B.; RAMOS, Cristiane S. *Hemigrammus arua*, a new species of characid (Characiformes: Characidae) from the lower Amazon, Brazil. *Neotropical Ichthyology*, São Paulo, v. 7, n. 2, p. 153–160, 2009.

LINNAEUS, C. (1753). *Species plantarum*, exhibentes plantas rite cognitatas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Holmiae [Stockholm]: **Impensis Laurentii Salvii**.

LINNAEUS, C., 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. Impensis Direct. **Laurentii Salvii**, Holmiae. 824 p.

LOPES, G. A. M. (2009). Sistemática do gênero *Ctenobrycon* Eigenmann, 1908 (Characiformes: Characidae) através da combinação de caracteres morfológicos e moleculares. Dissertação de Mestrado, **Universidade Estadual Paulista**.

- MACHADO, C. D. B., Ishizuka, T. K., Freitas, P. D., De Valiati, V. H., & Galetti, P. M. (2016). DNA barcoding reveals taxonomic uncertainty in *Salminus* (Characiformes). **Systematic Biodiversity**, 15, 372-382. <https://doi.org/10.1080/14772000.2016.1254390>
- MACLEOD, Norman. Morphometrics: History, development methods and prospects. *动物分类学报*, v. 42, n. 1, p. 4-33, 2017.
- MALABARBA, M. C. Fossil record and timing of characid diversification in South America. In: MALABARBA, L. R. et al. (Eds.). *Phylogeny and classification of Neotropical fishes*. Porto Alegre: **Edipucrs**, 1998. p. 107-123.
- MALABARBA, M. C. *Lignobrycon myersi*, a fossil characid (Teleostei: Ostariophysi) from the Tremembé Formation, Eocene-Oligocene of Brazil. **Comptes Rendus de l'Académie des Sciences - Series III**, v. 321, n. 4, p. 377-383, 1998.
- MANKTELOW, Mariette. History of taxonomy. Lecture from Dept. of Systematic Biology, **Uppsala University**, v. 29, 2010.
- MAYR, E. (1969). *Animal Species and Evolution*. **Harvard University Press**.
- Mayr, E. (1982) *The Growth of Biological Thought*. **Belknap P. of Harvard U.P**, Cambridge (Mass.).
- MAYR, E. , & Ashlock, P. D. (1991). *Principles of Systematic Zoology* (2nd ed.). **McGraw-Hill**.
- MC GEE, M. D., Borstein, S. R., Neutelings, G., et al. (2016). Accelerated diversification of cichlid fishes in African lakes using ultraconserved elements. **Proceedings of the Royal Society B: Biological Sciences**, 283(1830). <https://doi.org/10.1098/rspb.2016.1067>
- MELO, Bruno F. et al. Cryptic species in the Neotropical fish genus *Curimatopsis* (Teleostei, Characiformes). **Zoologica Scripta**, v. 45, n. 6, p. 650-658, 2016.
- MELO, B. F., et al. (2021). Oligocene Radiations and Characoidei Diversification. **Journal of Systematic Biology**, 70(5), 812-827.
- MELO, Bruno F. et al. Phylogenomics of Characidae, a hyper-diverse Neotropical freshwater fish lineage, with a phylogenetic classification including four families (Teleostei: Characiformes). **Zoological Journal of the Linnean Society**, v. 202, n. 1, p. zlae101, 2024.
- MINH, B. Q. et al. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. **Molecular Biology and Evolution**, v. 37, n. 5, p. 1530-1534, 2020. DOI: .
- MIRANDE, J. M. (2019). Morphology, molecules and the phylogeny of Characidae (Teleostei, Characiformes). **Cladistics**, 35(3), 282-300.
- MULLIS, K. , & Faloona, F. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. **Methods in Enzymology**, 155, 335-350. [https://doi.org/10.1016/0076-6879\(87\)55023-6](https://doi.org/10.1016/0076-6879(87)55023-6)
- OKUMURA, Mercedes; ARAUJO, Astolfo GM. Archaeology, biology, and borrowing: A critical examination of Geometric Morphometrics in Archaeology. **Journal of Archaeological Science**, v. 101, p. 149-158, 2019.
- OTA, Rafaela P. ; LIMA, Flávio CT; PAVANELLI, Carla S. A new species of *Hemigrammus* Gill, 1858 (Characiformes: Characidae) from the rio Madeira and rio Paraguai basins, with a redescription of *H. lunatus*. **Neotropical Ichthyology**, v. 12, p. 265-279, 2014.
- PAGEL, M. D. (1999). Inferring the historical patterns of biological evolution. **Nature**. 401:877-884.
- PAGEL M, Meade A, Barker D. Bayesian estimation of ancestral character states on phylogenies. **Systematic biology**. 2004 Oct 1;53(5):673-84.
- PARK, P. J., Aguirre, W. E., Spikes, D. A., & Miyazaki, J. M. (2013). Landmark-Based Geometric Morphometrics:

What Fish Shapes Can Tell Us about Fish Evolution. **Proceedings of the Association for Biology Laboratory Education**, 34, 361-371.

PATERSON,C. (1988). Homology in classical and molecular biology. **Molecular Biology and Evolution**, 5(6), 603-625.

PEARSON, Karl. LIII. On lines and planes of closest fit to systems of points in space. **The London, Edinburgh, and Dublin philosophical magazine and journal of science**, v. 2, n. 11, p. 559-572, 1901.

PEREIRA,L. H. G., Hanner, R., Foresti, F., & Oliveira, C. (2013). Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? **BMC Genetics**, 14:20. <https://doi.org/10.1186/1471-2156-14-20>

PITCHER,T. J.; PARRISH, J. K. Functions of shoaling behaviour in teleosts. In: PITCHER, T. J. (ed.). Behaviour of Teleost Fishes. 2. ed. **London: Chapman & Hall**, 1993. p. 363–439.

PÉLABON,C. , Firmat, C., Bolstad, G. H., Voje, K. L., Houle, D., Cassara, J., & Hansen, T. F. (2014). Evolution of morphological allometry. **Annals of the New York Academy of Sciences**, 1320(1), 58-75.

RAMBAUT,A. et al. Tracer v1.7: Posterior summarization in Bayesian phylogenetics. **Systematic Biology**, v. 67, n. 5, p. 901–904, 2018. DOI: 10.1093/sysbio/syy032.

RAMIREZ,J. L., et al. (2017). Revealing hidden diversity of the underestimated neotropical ichthyofauna: DNA Barcoding in the recently described genus Megaleporinus (Characiformes: Anostomidae). **Frontiers in Genetics**, 8(149), 1-11.

REIS,R. E., Albert, J. S., Di Dario, F., Mincarone, M. M., Petry, P., & Rocha, L. A. (2016). Fish Biodiversity and Conservation in South America. **Journal of Fish Biology**, 89, 12-47.

REVELL,L. J. phytools: an R package for phylogenetic comparative biology (and other things). **Methods in Ecology and Evolution**, v. 3, n. 2, p. 217–223, 2012. DOI: .

RICE,W. R. (1989). Analyzing tables of statistical tests. **Evolution**, 43(1), 223-225.

ROHLF,F. J. (2008). tpsUtil, file utility program (version 1.40). **Department of Ecology and Evolution, State University of New York at Stony Brook**.

ROHLF,F. J., & Slice, D. E. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. **Systematic Zoology**, 39(1), 40-59.

ROHLF,F. J. (2006). TPSDIG2 for Windows version 2.10. Available at: <http://life.bio.sunysb.edu/morph/index.html> (Accessed on: October 11, 2023).

ROHLF,F. J. On applications of geometric morphometrics to studies of ontogeny and phylogeny. **Systematic Biology**, v. 47, n. 1, p. 147–158, 1998.

ROHLF, F. J.; MARCUS, L. F. A revolution morphometrics. **Trends in ecology & evolution**, v. 8, n. 4, p. 129-132, 1993.

ROTH, V. L.; Mercer, J. M. Morphometrics in development and evolution. **American zoologist**, v. 40, n. 5, p. 801-810, 2000.

ROXO, F. F. et al. Phylogenomic reappraisal of the Neotropical catfish family Loricariidae (Teleostei: Siluriformes) using ultraconserved elements. **Molecular phylogenetics and evolution**, v. 135, p. 148-165, 2019.

SCLATER, P. L. On the general geographical distribution of the members of the class Aves. **Zoological Journal of the Linnean Society**, v. 2, n. 7, p. 130-136, 1858.

SHELTON,C. , et al. (2014). Three-dimensional reconstruction of biological specimens from serial section images. **Nature Protocols**, 9(7), 1870-1886. <https://doi.org/10.1038/nprot.2014.123>

- SIDLAUSKAS, B. (2008). Continuous and arrested morphological diversification in sister clades of characiform fishes: A phylomorphospace approach. **Evolution**, 62(12), 3135–3156.
- SIDLAUSKAS, B. L. et al. Molecular phylogenetics, a new classification, and a new genus of the Neotropical fish family Anostomidae (Teleostei: Characiformes). **Neotropical Ichthyology**, v. 23, n. 1, p. e240076, 2025.
- SIDLAUSKAS, B. L., Mol, J. H., & Vari, R. P. (2011). Dealing with allometry in linear and geometric morphometrics: A taxonomic case study in the *Leporinus cylindriformis* group (Characiformes: Anostomidae) with description of a new species from Suriname. **Zoological Journal of the Linnean Society**, 162(1), 103–130.
- SLICE, D. E. Landmark coordinates aligned by Procrustes analysis do not lie in Kendall's shape space. **Systematic Biology**, v. 50, n. 1, p. 141–149, 2001.
- SLICE, Dennis E. Geometric morphometrics. **Annu. Rev. Anthropol.**, v. 36, n. 1, p. 261–281, 2007.
- SOUZA, C. S. et al. Phylogenomic analysis of the Neotropical fish subfamily Characinae using ultraconserved elements. **Molecular Phylogenetics and Evolution**, v. 168, p. 107396, 2022.
- STRICKLAND, H. E., et al. (1842). Report of a committee appointed to consider the rules of zoological nomenclature. **London: British Association for the Advancement of Science**.
- THOMPSON, D. W. (1917). On Growth and Form. Cambridge University Press.
- THIMÓTHEO, M. G. T. Osteologia de *Dastilbe crandalli* Jordan, 1910 (Ostariophysi, Gonorynchiformes) da formação Crato, Cretáceo inferior da Bacia do Araripe, com uma discussão sobre a história taxonômica do gênero. 2025. Dissertação (Mestrado em Zoologia) – **Instituto de Biociências de Botucatu, Universidade Estadual Paulista (UNESP)**, Botucatu. 2025.
- TOLEDO-PIZA, M. et al. Checklist of the species of the Order Characiformes (Teleostei: Ostariophysi). **Neotropical Ichthyology**, v. 22, n. 1, p. e230086, 2024.
- VOGEL, S. Life in moving fluids: the physical biology of flow. 2. ed. **Princeton: Princeton University Press**, 1994.
- WAGNER, G. P.; ALTENBERG, Lee. Perspective: complex adaptations and the evolution of evolvability. **Evolution**, v. 50, n. 3, p. 967–976, 1996.
- WAINWRIGHT, P. C.; RICHARD, B. A. Scaling the feeding mechanism of largemouth bass (*Micropterus salmoides*): kinematics of prey capture. **Journal of Experimental Biology**, v. 198, p. 419–433, 1995.
- WAINWRIGHT, P. C. et al. Many-to-one mapping of form to function: a general principle in organismal design? **Integrative and Comparative Biology**, v. 35, n. 3, p. 283–291, 1995.
- WAKELING, J. M. Fast-starting fish are not slow: variability of performance and motor control during escape responses. **Journal of Experimental Biology**, v. 209, p. 2622–2634, 2006.
- WEBB, P. W. Body form, locomotion and foraging in aquatic vertebrates. **American Zoologist**, v. 24, n. 1, p. 107–120, 1984.
- WEISS, F. E. M. et al. Phylogenetic relationships of *Paleotetra*, a new characiform fish from the Eocene-Oligocene of south-eastern Brazil. **Zoological Journal of the Linnean Society**, v. 165, n. 4, p. 848–863, 2012.
- WEISS, F. E. M. et al. Phylogenetic relationships of *Paleotetra*, a new characiform fish (Ostariophysi) with two new species from the Eocene–Oligocene of south-eastern Brazil. **Journal of Systematic Palaeontology**, v. 10, n. 1, p. 73–86, 2012. DOI: 10.1080/14772019.2011.565081.
- WEITZMAN, M. , & Weitzman, S. H. (2003). Lebiasinidae (pencil fishes). In: Reis, R. E., Kullander, S. O., & Ferraris, C. J. Jr. (Eds.), Checklist of the freshwater fishes of South and Central America. **EDIPUCRS**, Porto Alegre, Brazil, pp. 241–251.

WHEELER, D. A., BARDEEN, C., et al. (2008). The complete genome of an individual by massively parallel DNA sequencing. **Nature**, 452, 872-876. <https://doi.org/10.1038/nature06884>

WILEY, Edward Orlando; LIEBERMAN, Bruce S. Phylogenetics: theory and practice of phylogenetic systematics. **John Wiley & Sons**, 2011.

XIMENES, Aline Mourão. Diversidade de espécies de *Geophagus* Heckel, 1840 (Cichliformes: Cichlidae) da bacia amazônica utilizando abordagem genômica e de DNA mitocondrial. 2023.

YANG, Z. ; RANNALA, B. Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. **Molecular Biology and Evolution**, v. 23, n. 1, p. 212–226, 2006. DOI: 10.1093/molbev/msj024.

ZELDITCH, M. L., SWIDERSKI, D. L., & SHEETS, H. D. (2012). Geometric Morphometrics for Biologists: A Primer (2nd ed.). **Academic Press**, London.

ZELDITCH, M. L.; SWIDERSKI, D. L.; SHEETS, H. D.; FINK, W. L. Geometric Morphometrics for Biologists: A Primer. 2. ed. **London: Elsevier Academic Press**, 2012.

ZELDITCH, M., SWIDERSKI, D.; SHEETS, H. David. Geometric morphometrics for biologists: a primer. **Academic Press**, 2012.

ZHANG, R. ; DRUMMOND, A. J. Improving the performance of Bayesian phylogenetic inference under relaxed clock models. **BMC Evolutionary Biology**, v. 20, n. 54, 2020. DOI: 10.1186/s12862-020-01627-8.