FishPhyloMaker: An R package to generate phylogenies for ray-finned fishes

Authors: Gabriel Nakamura1, 2, \*, Aline Richter1, Bruno E. Soares3

1 – Universidade Federal do Rio Grande do Sul, Departamento de Ecologia, Bento Gonçalves Avenue, 9500, CP

2 – INCT Ecology, Evolution, and Biodiversity Conservation

3 – Universidade Federal do Rio de Janeiro, Programa de Pós-Graduação em Ecologia

\*correspondence author: gabriel.nakamura.souza@gmail.com

# Highlights

* We provided the first automated procedure to check species names, construct phylogenetic trees and calculate Darwinian shortfalls for ray-finned fishes (Actinopterygii). This procedure is provided through an R package called FishPhyloMaker.
* This tool provides functions to assemble phylogenies with a fast and reproductible method, allowing its use and replicability by specialists and non-specialists in the group.
* The tool also provide an interactive insertion procedure, that gives more flexibility to the user when compared with other existant tools that construct phylogenetic trees for other highly speciose groups.
* The rationale of the provided algorithm can be extended in future studies to be used in other groups of organisms.

# Abstract

Phylogenies summarizes evolutionary information that is essential in the investigation of ecological and evolutionary causes of diversity patterns. They allow investigating hypotheses from trait evolution to the relationship between evolutionary diversity and ecosystem functioning. However, obtaining a comprehensive phylogenetic hypothesis can be difficult for some groups, specially those with high number of species, that is the case for fishes, particularly, tropical assemblages. The lack of species in phylogenetic hypothesis, called Darwinian shortfalls, can hinder ecological and evolutionary studies involving this group. To tackle this problem we developed FishPhyloMaker, an R package that facilitates the generation of phylogenetic trees, even for a great number of species, through a reliable and reproducible procedure. The package adopts well-known rules of insertion based on cladistic hierarchy, that allows its used by specialist and non-specialists in the group. We exemplified the use of FishPhyloMaker package by constructing complete phylogenies for fishes inhabiting the four freshwater ecoregions of the world. Furthermore we proposed a new method to calculate Darwinian shortfalls and showed how to map this information for all major freshwater drainages of the world. FishPhyloMaker will expand the range of evolutionary and ecological questions that can be addressed using ray-finned fishes as study models by provide an ease and reliable way to obtain comprehensive phylogenies.

# Introduction

Phylogenies have been widely explored in ecology in the last decades due to the development of theoretical frameworks, numerical methods, and software (*e.g.,* Webb et al. 2008; Felsenstein 1985). The research agenda in ecology and evolution encompasses phylogenetic approaches from organismal to macroecological-scale, including trait evolution, invasion ecology, metacommunity ecology, and ecosystem functioning (Cavender-Bares et al., 2009). Hence, comprehensive phylogenetic trees must be available to address those topics.

Well-established phylogenies for most of known species are available for some groups, such the terrestrial vertebrates (birds (Jetz et al., 2012), mammals (Upham et al., 2019), amphibians (Jetz and Pyron, 2018), squamates (Tonini et al., 2016), sharks (Stein et al., 2018)) and plants (Magallón et al., 2015), which have also powerfull tools to generate phylogenetic trees for local/regional pools of species (*e.g.,* Webb & Donoghue 2005 for mammals and plants; Jin & Qian 2019 for plants, to the others see http://vertlife.org/phylosubsets/). Inversely, available phylogenies for bony fishes (Betancur et al., 2017; Rabosky et al., 2018) display issues related to the taxonomic position of some clades (e.g., non-monophyletic groups) and the lack of species representativeness. The latter issue hampers answering some questions on the ecology and evolution of bony fishes by generating inaccuracy to estimates of phylogenetic signal, trait evolution, and phylogenetic diversity (Seger et al. 2010; Boettiger et al., 2012a), or even impeding their calculation.

Ray-finned fishes (Actinopterygii) exhibit a complex evolutionary history and high ecological diversity (Albert et al., 2020), making them an interesting group to address questions in the interface of ecology and evolution (*e.g.*, Roa-Fuentes et al. 2019; Nakamura et al. 2020). The difficulty in obtaining phylogenetic information can hinder our efforts to understand fish ecology and evolution. Additionally, some issues as the lack of phylogenetic information for species, *i.e*., Darwinian shortfalls, end up being restricted to few lineages (*e.g.*, Freitas et al., 2021), which impedes the mapping of the relative demand of additional efforts in specific regions or clades. when did “by hand” proceduresAn alternative would be the use of to ,it comprehensive by using the information from cladistic hierarchy, as suggested by Diniz-Filho et al (2013)

In order to tackle the problem of obtention of comprehensive fish phylogenies in a reliable and reproducible way, we developed the FishPhyloMaker, a freely available R package that facilitate the obtention of phylogenetic trees for ray-finned fishes. FishPhyloMaker automates the insertion procedure of species in the most comprehensive phylogeny (Rabosky et al., 2018) of ray-finned fishes following their taxonomic hierachy. We illustrated how FishPhyloMaker package solve the problem of obtaintion of comprehensive phylogenies by constructing phylogenetic trees for species inhabiting the four major freshwater ecoregions (Afrotropics, Australasia, Indo-malay, Neartic and Neotropics, sensu Tedesco et al., (2017)). Further, we developed a new method to quantify the Darwinian shortfalls and illustrate the use of this method by mapping the Darwinian shortfalls of all the major word freshwater drainages. Our package overcomes the main problems associated with manually building phylogenies for ray-finned fishes by following a specific and documented procedure and reducing the manual labor in large phylogenies.

**Methods**

# Inside the Fish(PhyloMaker): an overview of the package

FishPhyloMaker is a freely-available R package containing three main functions, FishTaxaMaker() and FishPhyloMaker(). Below, we describe these functions and how they can be used to obtain phylogenetic trees, highlighting the input data, intermediate steps, and output objects. Brief descriptions of the package functions are available in Table 1.

*FishTaxaMaker()*

FishTaxaMaker() checks the validity of species names provided by the user and prepares a formatted data frame to be used in the FishPhyloMaker() function. The input data must be a string vector or a data frame containing a list of species from the regional pool or an occurrence matrix (sites x species). The genus and specific epithet (or subspecies) must be separated by underline (e.g., *Genus*\_*epithet*). The function first classifies the provided species names as valid or synonymies based on Fishbase (Froese & Pauly, 2006) by using the *rfishbase* package (Boettiger et al., 2012b). A new column summarizes names initially valid and the current valid names substituting identified synonymies. Unknown species to Fishbase are printed in the command line, and the user must manually inform the Family and the Order of the species. The output of the function is a list containing three elements: 1) a data frame displaying the taxonomic information (Valid name, Subfamily, Family, Order, Class, and SuperClass) for each provided species; 2) a data frame displaying the taxonomic information (Species, Family, and Order), only for the valid species; 3) a character vector displaying the species names not found in Fishbase.

Table 1: Functions presented in the package FishPhyloMaker and their descriptions.

|  |  |
| --- | --- |
| **Function** | **Description** |
| *FishTaxaMaker*() | Checks species names according to Fishbase and prepares the species list for the other functions in the package. |
| *whichFishAdd*() | Identifies the species already included in the mega-tree and in which taxonomic level each remaining species will be inserted. |
| *FishPhyloMaker*() | Builds the phylogeny and may return a data frame identifying step-by-step the performed insertions. |
| *Darwinian\_deficit*() | Calculates the Darwinian shortfall for the provided species list through a Phylogenetic Diversity (PD Faith (1992)) ratio |

*FishPhyloMaker()*

This is the core function of the package. This function builds a phylogenetic hypothesis for the provided species list by inserting in and pruning species from the Rabosky's et al. (2020) phylogenetic tree (Figure 1) downloaded by the fishtreeoflife R package (Chang et al. 2019). This phylogeny is the most up-to-date and comprehensive phylogenetic hypothesis for ray-finned fishes.

The input for *FishPhyloMaker* function can be the second element in the list returned by *FishTaxaMaker* function or a manually constructed data frame with the same configuration (species, family, and order names for each taxon). The function also contains three logical arguments: insert.base.node, return.insertions and progress.bar. These three arguments are set by default as FALSE, TRUE, and TRUE, respectively, and allow the user to choose if the species must be inserted at the base node of families/orders, if the insertions made by each species must be showed as output results and if a progress bar must be showed in the console.

The function works sequentially, first identifying which of the provided species are in the backbone phylogenetic tree. If all of them are already present in the backbone tree, the function returns a pruned one. If any of the provided species is not in the backbone tree, the function performs a four-level insertion routine. First, species from genera already included in the backbone tree are inserted as polytomies at the most recent ancestral node that links all congeneric species (or as the sister species of the only species representing a genus in the backbone tree, as shown in *i* in Figure 1). Second, species not inserted in the previous step are then inserted at the family level by an interactive procedure using a returned list of all the genera within the same family of the target species. The user has the option to insert the target species as a sister taxon to a genus (*ii* in Figure 1, option 1, near to *Loricaria* genus), between two genera (*ii* in Figure 1, option 2, between genus *Loricaria* and *Hypostomus*), or at the node of the family (*ii* in Figure 1, option 3). If the user enters a single genus from the list, the function splits its branch and inserts the target species as a sister taxon (option 1). If the user enters two genera separated by a blank space, the function inserts the target species as a polytomy at the most recent node that links the selected genera (option 2). If the user enters the family name, the function inserts the target species at the family node as a polytomy (option 3). Third, if any remaining species can now be inserted at the genus level, the function repeats the first procedure but records it as a Congeneric family-level insertion (*iii* in Figure 1). Fourth, remnant species are inserted at the order level following similar to the second step by an interactive procedure using a returned list of all the families within the order of the target species. Hence, the user may specify a family to insert the target species as sister taxon (option 1), two families to insert it as a polytomy at the most recent node linking them (option 2), or the order to insert it as a sister taxon (option 3). The function will not perform insertions steps beyond the order level because it would add too much uncertainty to the phylogenetic tree.

Setting the argument insert.base.node as TRUE automatically inserts the target species from the second and fourth steps in the family and order nodes, respectively. This setting facilitates the insertion of a large number of species or species with the phylogenetic position unknown. The default output is a list with two objects: (i) the pruned tree including only the provided species list (Final tree in Figure 1); (ii) a data frame identifying if each provided species was initially present in the backbone tree, in which step it was inserted, or not inserted at all.



Figure 1: Schematic representation of insertion and subsetting procedure performed by the FishPhyloMaker() function. Here we used a hypothetical phylogeny containing ten species and four families (silhouettes inside the tree) as the backbone phylogeny. Step (i) represents the congeneric level of insertion. Step (ii) represents the three options that the user may choose in the Family-level round of insertions (Option 1 – near to a genus; Option 2 – between two genera; Option 3 – at the family node). (iii) represents the congeneric insertions at the family level, and, finally, the final pruned tree containing only the species of interest.

*Darwinian\_deficit()*

This function allows the calculation of a measure of Darwinian shortfalls. This calculation is made trhough the Equation 1:

Equation 1

In this function, PDinserted represented the sum of the branch lengths of species that was presented in the phylogenetic tree before the insertion procedure. PDpresented in tree represented the sum of branch lengths of the species that was added in the phylogenetic tree during the insertion procedure. Equation 1 reaches 1 when all the species that compose the phylogentetic tree was inserted, and is near to zero when the majority of species was already present in the tree before the insertion procedure. To calculate the Darwinian shortfall throught *Darwinian\_deficit* function the user must provide a phylogenetic tree and a table of insertions, both obtained from *FishPhyloMaker* function.

*Illustrating the use of FishPhyloMaker package*

We provide an example of the usage of the *FishPhyloMaker* package by creating a phylogenetic trees using a global dataset of freshwater fishes inhabiting 3119 drainage basins that cover more than 80% of earth surface (Tedesco et al., 2017). This dataset allowed in-depth investigation on the global patterns of species distribution and their evolutionary determinants (*e.g.*, Miller & Román-Palácios, 2021). We used the data from Tedesco et al. (2017) to construct phyologenetic trees for the four major freshwater ecoregions of the world based on species richness: Afrotropics, Indo-Malay, Neartic and Neotropics. Moreover, we used this same dataset to demonstrate how to map the Darwinian shortfalls, calculated following Equation 1 and *Darwinian\_deficit* function, for all the drainage basins in the Tedesco et al (2017) dataset.

First, the user must download the package from the online GitHub repository, using the following command line:

devtools::install\_github("GabrielNakamura/FishPhyloMaker", ref = "main", build\_vignettes = TRUE)

We recommended that the user update all package requested, to avoid errors related with package versions. We prepared the fish occurrence by checking the validity of its names by using the function *FishTaxaMaker*. The occurrence matrix encompassed 2478 species, from which 2477 were valid names. With valid names returned from *FishTaxaMaker* function, we applied the *FishPhyloMaker* function separately for each pool of species in each ecoregions considered in this analysis (Figure 2). For simplicity, and reprodutibility we set the argument insert.base.node as TRUE, thus, inserting all species at the base node of its corresponding family and order. We also set the argument return.insertions = TRUE, so that we can used the information regarding the insertion of each species in the *Darwinian\_deficit* function to calculated Darwinian shortfalls for all freshwater basins of the world that present, at least, three species in the Tedesco et al. (2017) dataset. We used only the branch lengths of species added as congenetic species to calculate the Darwinian shortfall, but it is worth to highlight that the function *Darwinian\_deficit* allows to include other levels of phylogenetic insertion, like family and order insertions.

All the codes need to fully reproduce this analysis is provided at GitHub (GabrielNakamura/MS\_FishPhyloMaker). Further analysis exploring the usage of the package is available at https://gabrielnakamura.github.io/FishPhyloMaker/index.html.

**Results**

The entire insertion procedure lasted approximately two hours using one core from a computer machine with an i5 processor. A total of 821 species were inserted, with the Afrotropics exhibiting the largest number of insertions (359 from 767, Figure 2). On the contrary, Nartic was the ecoregion that presented the least number of species inserted, being only six species inserted at the genus level (congeneric species), which represent only 2.7% of missing species in phylogenetic tree for this region. Indo-Malay and Neotropic presented 30.3% and 30% respectively of species inserted at all levels of taxonomic hierarchy considered (genus, family and order).

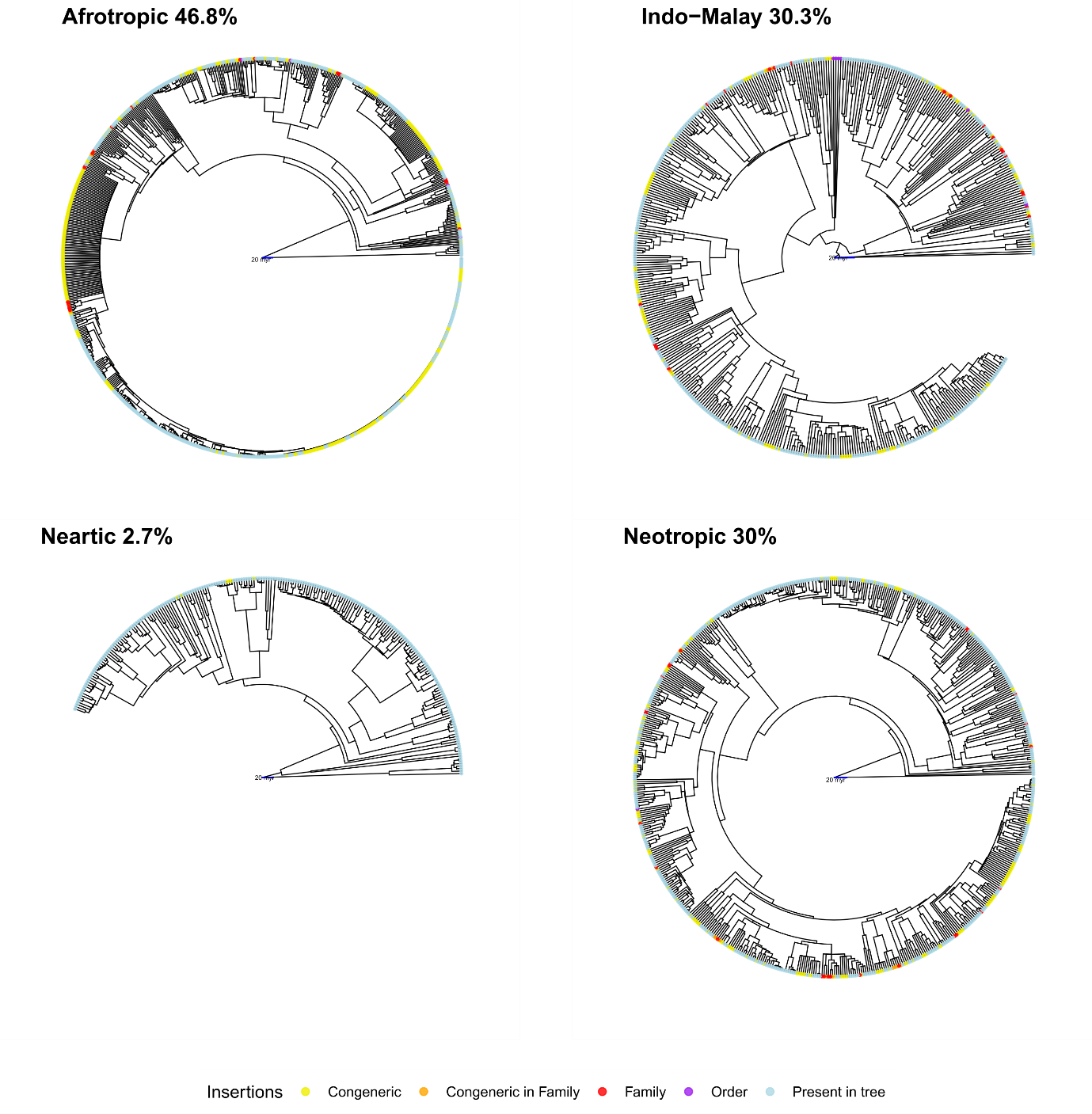


Figure 2: Phylogenetic trees generated with the FishPhyloMaker package for freshwater fishes inhabiting four ecoregions (Afrotropics, Indo-Malay, Nearctic, and Neotropic). The colored tip-points indicate if species were present in the backbone tree (Present in tree) or at which level they were inserted. The percentages of insertions over the total number of species for each ecoregion are shown.

We observed that tropical regions exhibited larger values of Darwinian shortfalls, while northest sites had the lower values of shortfalls (Figure 3). Specifically, the highest values of Darwinian shortfalls was found in Afrotropics, with some drainages presenting any species in the Rabosky´s phylogenetic tree, and Neotropics, particularly the Amazon basin with approximately 0.75 of total branch lengths represented by species occurring in Amazon basin is absent of Rabosky´s phylogeny.

**Discussion**

, efficients for insertion of absent species, that allows the calculation of, until as we know, the first quantitative measure of Darwinian shortfall for freshwater fish assemblages

Whereas Phylomatic allows the insertion of absent species only as congeneric or at the base node corresponding to the family of the focal species (Webb and Donoghue, 2005), FishPhyloMaker delivers more option to the user since it allow to perform a interactive procedure of insertion that can be fully reproducible since the insertions can be registred in an R script, providing, at the same time, more flexibility and the same level of reproducibility of other algorithms designed for similar propouses (e.g. Jin and Qian, 2019).

Despite tropical region are recognized as biodiversity hotspots, the regions concentrate higher values of Darwinian shortfalls than temperate regions. This loss could lead a bias in the evaluation of evolutionary history or macroecological patterns for fish assemblages in tropical region. Generally, the existence of the lack in biodiversity knowledge is related with the rate of formation of new species, which we can not describe at the same speed (Hortal et al., 2015), beside that, richest regions need for more sampling effort and research investiment to access a reliable representativeness of total biodiversity.

*Limitations and possible applications*

Future developments of the package should consider the Catalog of Fishes (Fricke & Eschemeyer, 2021) to improve the nomenclature checking procedures. Despite Fishbase being a widely used database to check for the taxonomic classification of fishes, it may present delays in updating taxonomic information because it is not its primary purpose. Inversely, the Catalog of Fishes is an authoritative taxonomic list frequently updated.

An inherent limitation of the phylogenetic hypothesis produced by FishPhyloMaker is the large number of polytomies resulting from the insertion procedures. We recommend that users directly assess how the phylogenetic uncertainty affects further analysis when not using a fully solved phylogenetic tree (Martins et al., 2013).

These limitations do not preclude the package applicability for studies in phylogenetic community ecology since synthesis phylogenies do not significantly impact phylogenetic diversity indices (Li et al., 2019). Moreover, this is the only automated tool able to provide a complete phylogenetic tree that can easily handle large datasets. FishPhyloMaker can be relevant for addressing several critical questions in ecology and evolution by facilitating the obtention of phylogenetic hypotheses for local pools of ray-finned fishes. This facilitation can be essential for regions with a large gap in the phylogenetic knowledge of fishes, such as the Neotropical region (Albert et al., 2020). Such phylogenetic hypotheses allow understanding how ecological traits evolved or how the current and past environmental conditions selected the lineages in different areas.

At larger scales, biogeographical studies are usually restricted to one or a few lineages due to the availability of molecular phylogenies. The FishPhyloMaker package facilitates large-scale investigations on the biogeographic history of the most diverse group of vertebrates on Earth, the Actinopterygians, helping us understand the processes that drive this high diversity. Finally, we can map where the lack of phylogenetic information is the most critical once the function returns the insertion-level of species. This information can directly elucidate the patterns of the Darwinian shortfalls for ray-finned fishes. Therefore, we expect that the FishPhyloMaker package reduces the gaps and barriers to addressing ecological and evolutionary questions due to the difficulty or lack of a reliable phylogenetic hypothesis for local and regional pools of ray-finned fishes.

**Contributions**

**Acknowledgements**

GN is member of the National Institutes for Science and Technology (INCT) in

Ecology, Evolution and Biodiversity Conservation, supported by MCTIC/CNPq (proc.

465610/2014-5). BES and AR are grateful to FAPERJ and CAPES for their postdoctoral and doctoral grants, respectively. The Brazilian science resists.

# References

Albert, J.S., Tagliacollo, V.A., Dagosta, F., 2020. Diversification of Neotropical Freshwater Fishes. Annu. Rev. Ecol. Evol. Syst. 51, 27–53. https://doi.org/10.1146/annurev-ecolsys-011620-031032

Betancur, R.R., Wiley, E.O., Arratia, G., Acero, A., Bailly, N., Miya, M., Lecointre, G., Ortí, G., 2017. Phylogenetic classification of bony fishes. BMC Evol. Biol. 17, 1–40. https://doi.org/10.1186/s12862-017-0958-3

Boettiger, C., Coop, G., Ralph, P., 2012. Is your phylogeny informative? Measuring the power of comparative methods. Evolution (N. Y). 66, 2240–2251. https://doi.org/10.1111/j.1558-5646.2011.01574.x

Cavender-Bares, J., Kozak, K.H., Fine, P.V.A., Kembel, S.W., 2009. The merging of community ecology and phylogenetic biology. Ecol. Lett. 12, 693–715. https://doi.org/10.1111/j.1461-0248.2009.01314.x

Chang, J., Rabosky, D.L., Smith, S.A., Alfaro, M.E., 2019. An r package and online resource for macroevolutionary studies using the ray-finned fish tree of life. Methods Ecol. Evol. 10, 1118–1124. https://doi.org/10.1111/2041-210X.13182

Diniz-Filho, J.A.F., Loyola, R.D., Raia, P., Mooers, A.O., Bini, L.M., 2013. Darwinian shortfalls in biodiversity conservation. Trends Ecol. Evol. 28, 689–695. https://doi.org/10.1016/j.tree.2013.09.003

Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biol. Conserv. 61, 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125, 1–15. https://doi.org/0003-0147/85/2501-0001

Freitas, T.M. da S., Stropp, J., Calegari, B.B., Calatayud, J., De Marco, P., Montag, L.F. de A., Hortal, J., 2021. Quantifying shortfalls in the knowledge on Neotropical Auchenipteridae fishes. Fish Fish. 22, 87–104. https://doi.org/10.1111/faf.12507

Hortal, J., De Bello, F., Diniz-Filho, J.A.F., Lewinsohn, T.M., Lobo, J.M., Ladle, R.J., 2015. Seven Shortfalls that Beset Large-Scale Knowledge of Biodiversity. Annu. Rev. Ecol. Evol. Syst. 46, 523–549. https://doi.org/10.1146/annurev-ecolsys-112414-054400

Jetz, W., Pyron, R.A., 2018. The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. Nat. Ecol. Evol. 2, 850–858. https://doi.org/10.1038/s41559-018-0515-5

Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K., Mooers, A.O., 2012. The global diversity of birds in space and time. Nature 491, 444–448. https://doi.org/10.1038/nature11631

Jin, Y., Qian, H., 2019. V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. Ecography (Cop.). 42, 1353–1359. https://doi.org/10.1111/ecog.04434

Li, D., Trotta, L., Marx, H.E., Allen, J.M., Sun, M., Soltis, D.E., Soltis, P.S., Guralnick, R.P., Baiser, B., 2019. For common community phylogenetic analyses, go ahead and use synthesis phylogenies. Ecology 100, 1–15. https://doi.org/10.1002/ecy.2788

Magallón, S., Gómez-Acevedo, S., Sánchez-Reyes, L.L., Hernández-Hernández, T., 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. New Phytol. 207, 437–453. https://doi.org/10.1111/nph.13264

Martins, W.S., Carmo, W.C., Longo, H.J., Rosa, T.C., Rangel, T.F., 2013. SUNPLIN: Simulation with Uncertainty for Phylogenetic Investigations. BMC Bioinformatics 14. https://doi.org/10.1186/1471-2105-14-324

Nakamura, G., Vicentin, W., Súarez, Y.R., Duarte, L., 2020. A multifaceted approach to analyzing taxonomic, functional, and phylogenetic β‐diversity. Ecology. https://doi.org/10.1002/ecy.3122

Rabosky, D.L., Chang, J., Title, P.O., Cowman, P.F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T.J., Coll, M., Alfaro, M.E., 2018. An inverse latitudinal gradient in speciation rate for marine fishes. Nature 559, 392–395. https://doi.org/10.1038/s41586-018-0273-1

Roa-Fuentes, C.A., Heino, J., Cianciaruso, M. V., Ferraz, S., Zeni, J.O., Casatti, L., 2019. Taxonomic, functional, and phylogenetic β-diversity patterns of stream fish assemblages in tropical agroecosystems. Freshw. Biol. 64, 447–460. https://doi.org/10.1111/fwb.13233

Roa-Fuentes, C.A., Heino, J., Zeni, J.O., Ferraz, S., Cianciaruso, M.V., Casatti, L., 2020. Importance of local and landscape variables on multiple facets of stream fish biodiversity in a Neotropical agroecosystem. Hydrobiologia 7. https://doi.org/10.1007/s10750-020-04396-7

Roquet, C., Thuiller, W., Lavergne, S., 2013. Building megaphylogenies for macroecology: Taking up the challenge. Ecography (Cop.). 36, 13–26. https://doi.org/10.1111/j.1600-0587.2012.07773.x

Stein, R.W., Mull, C.G., Kuhn, T.S., Aschliman, N.C., Davidson, L.N.K., Joy, J.B., Smith, G.J., Dulvy, N.K., Mooers, A.O., 2018. Global priorities for conserving the evolutionary history of sharks, rays and chimaeras. Nat. Ecol. Evol. 2, 288–298. https://doi.org/10.1038/s41559-017-0448-4

Tedesco, P.A., Beauchard, O., Bigorne, R., Blanchet, S., Buisson, L., Conti, L., Cornu, J.F., Dias, M.S., Grenouillet, G., Hugueny, B., Jézéquel, C., Leprieur, F., Brosse, S., Oberdorff, T., 2017. Data Descriptor: A global database on freshwater fish species occurrence in drainage basins. Sci. Data 4, 1–6. https://doi.org/10.1038/sdata.2017.141

Tonini, J.F.R., Beard, K.H., Ferreira, R.B., Jetz, W., Pyron, R.A., 2016. Fully-sampled phylogenies of squamates reveal evolutionary patterns in threat status. Biol. Conserv. 204, 23–31. https://doi.org/10.1016/j.biocon.2016.03.039

Upham, N.S., Esselstyn, J.A., Jetz, W., 2019. Inferring the mammal tree: Species-level sets of phylogenies for questions in ecology, evolution, and conservation. PLOS Biol. 17, e3000494. https://doi.org/10.1371/journal.pbio.3000494

Webb, C.O., Ackerly, D.D., Kembel, S.W., 2008. Phylocom: Software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24, 2098–2100. https://doi.org/10.1093/bioinformatics/btn358

Webb, C.O., Donoghue, M.J., 2005. Phylomatic: Tree assembly for applied phylogenetics. Mol. Ecol. Notes 5, 181–183. https://doi.org/10.1111/j.1471-8286.2004.00829.x