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

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Highlights

• We provide the first automated procedure to check species names, construct

phylogenetic trees and calculate Darwinian shortfalls for ray-finned fishes

(Actinopterygii) by the R package FishPhyloMaker.

• This package provides functions to assemble phylogenies through a fast, reliable, and

reproducible method, allowing its use and replicability by specialists and non-

specialists in fish systematics.

• The package also provides an interactive procedure that gives more flexibility to the

user when compared with other existing tools that construct phylogenetic trees for

other highly speciose groups.

• The package includes a new method to compute Darwinian shortfalls for ray-finned

fishes, but the rationale of the provided algorithm can be extended in future studies to

be used in other groups of organisms

Abstract

Phylogenies summarize 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, especially those with a high number of species, that is the case for fishes,

particularly tropical ones. The lack of species in phylogenetic hypotheses, 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 through a reliable and reproducible procedure, even for a large number of

species. The package adopts well-known rules of insertion based on cladistic hierarchy,

allowing its use by specialists and non-specialists in fish systematics. We tested the reliability

of our algorithm in maintaining important properties of phylogenetic distances running a

sensitivity analysis. We also exemplified the use of the FishPhyloMaker package by

constructing complete phylogenies for fishes inhabiting the four richest freshwater ecoregions

of the world. Furthermore, we proposed a new method to calculate Darwinian shortfalls and

mapped this information for the 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, mainly in the field of community phylogenetics, by

providing an easy and reliable way to obtain comprehensive phylogenies. Further,

FishPhyloMaker presents the potential to be extended to other taxonomic groups that suffer

from the same difficulty in the obtention of comprehensive phylogenetic hypothesis.

Keywords: Phylogenies, community phylogenetics, Darwinian shortfall, gap-analysis.

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. Large

phylogenies were primarily developed by combining source-trees and published-trees (the

supertree approach), by concatenating different data matrices of systematic phylogenetic

characters to generate a single tree (the supermatrix approach), or by a mix of both

approaches (Haeseler, 2012; Smith et al., 2009).

Well-established phylogenies for most of the known species are available for some

groups, such as 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 also have powerful 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 ray-finned fishes by

generating inaccuracy in estimates of phylogenetic signal, trait evolution, and phylogenetic

diversity (Seger et al., 2013; 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, the lack of phylogenetic information for

species, *i.e.*, Darwinian shortfalls, is currently investigated in a few lineages (*e.g.*, Freitas et

al., 2021), which impedes the mapping of the relative demand of additional efforts needed in

entire regions or clades to uncover the phylogenetic history of fishes. This problem urges a

rapid solution in the context of the accelerated loss of species (Chase et al., 2020).

A short-term solution to tackle the Darwinian shortfall for ray-finned fishes would be

coupling the phylogenetic information with cladistic classification to produce comprehensive

phylogenies (Diniz-Filho et al., 2013). This solution is laborious and lacks reproducibility

when adding many species, and the specific steps are not precisely documented when did "by

hand" procedures (Webb et al., 2008). An alternative would be using molecular techniques to

generate comprehensive phylogenies (e.g. Pie et al., 2021). However, it demands high

expertise and high financial investment (Roquet et al., 2013), limiting factors for several

institutions. Therefore, automatizing the procedures of constructing comprehensive

phylogenies using the information from cladistic hierarchy, as suggested by Diniz-Filho et al

(2013), provides a more reliable, accessible, and short-term solution for evolutionary

ecologists. The technique produces reliable phylogenetic information for community

phylogenetics (Li et al., 2019).

In order to tackle the problem of obtention of comprehensive fish phylogenies in a

reliable and reproducible way, we developed the FishPhyloMaker. This freely available R

package facilitates 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 hierarchy. We illustrated how the

FishPhyloMaker package solves the problem of obtaining comprehensive phylogenies by

constructing phylogenetic trees for species inhabiting more than 3000 freshwater basins

globally (Tedesco et al., 2017). Further, we developed a new method to quantify the

Darwinian shortfalls, which we illustrate by mapping the Darwinian shortfalls for the

abovementioned basins. Finally, we performed a sensitivity analysis to evaluate how our

method preserves characteristics of the phylogenetic tree (pairwise distances among species

and evolutionary distinctiveness), even with a varying number of inserted taxa. 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

A stable version of FishPhyloMaker can be downloaded from the CRAN repository

(https://cran.r-project.org/web/packages/FishPhyloMaker/index.html), and a development

version is available at the GitHub repository

(https://github.com/GabrielNakamura/FishPhyloMaker). All analyses shown here were

performed using the development version of FishPhyloMaker.

FishPhyloMaker is a freely available R package containing three main functions,

FishTaxaMaker, *FishPhyloMaker,* and *PD_deficit*. Below, we describe the functions to

generate phylogenetic trees, highlighting the input data, intermediate steps, and output

objects. Brief descriptions of the package functions are available in Table 1.

FishTaxaMaker

The *FishTaxaMaker* function checks the validity of species names provided by the user and

prepares a formatted data frame for 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, 2000) 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 of these species.

If the user types a Family not recognized in the FishBase, the user is asked to check the

spelling and type the Order of this family. 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 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 backbone 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.
PD_deficit()	Calculates the Darwinian shortfall for the

provided species list through a Phylogenetic

Diversity (PD Faith (1992)) ratio

FishPhyloMaker

FishPhyloMaker is the core function of the package. This function builds a phylogenetic tree

for the provided species list by inserting in and pruning species from the Rabosky et al.,

(2018) 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 the *FishPhyloMaker* function can be the second element in the list

returned by the *FishTaxaMaker* (Taxon_data_FishPhyloMaker) 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 at the

base node of families/orders, if the insertions made by each species must be shown in the

output and if a progress bar must be shown in the console.

The function works sequentially, first identifying which of the provided species are in

the backbone phylogenetic tree (Rabosky et al., 2018). 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. In the case of *i* in Figure

1 the branch length is divided at half of its length and the species is inserted. 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 as a

sister taxon of this genus (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 attaches 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 by splitting the branch length of the congeneric species at

half of its length (*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

option facilitates the insertion of a large number of species or species with the unknown

phylogenetic position. 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. This data frame will flag each species with one of the six classification

based on the insertion procedure: 1 – Present in tree will indicate species that were already

present in the backbone tree; 2 – Congeneric insertion will indicate species that present at

least one species of the same genus in the backbone tree and was inserted as congeneric of

this species; 3 – Family insertion will indicate inserted species that did not present any

congeneric species at backbone tree, but had at least one species of the same family in

backbone tree; 4 – Congeneric at Family-level will indicate species that was added as

congeneric after another species of the same genus was inserted at the Family level; 5 – Order

insertion will indicate inserted species that did not presented any species of the same family

in the backbone tree and must be inserted near to an extant family or in node corresponding

to the order root in the backbone tree; 6 – not inserted will indicate species that did not

present any species of the same order in the backbone tree, therefore was not inserted due

their high uncertainty in the phylogenetic position.

8   Figure 1: Schematic representation of insertion and subsetting procedure performed by the FishPhyloMaker() function. Here we used a hypothetical phylogeny

9   containing ten species and four families (silhouettes inside the tree) as the backbone phylogeny. Step (i) represents the congeneric level of insertion. Step (ii)

0   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;

1   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

2   interest.

PD_deficit

The *PD_deficit* function calculates a measure of Darwinian shortfalls following

Equation 1:

$$PD_{inserted} \Big/ PD_{inserted} + PD_{present\ in\ tree} \qquad (1)$$

In this function, $PD_{inserted}$ is the sum of the branch lengths of species in the phylogenetic tree

before the insertion procedure. $PD_{present\ in\ tree}$ is the sum of branch lengths of the species

inserted in the tree. Therefore, the Darwinian deficit ranges from 0 (all species already

present in the backbone tree before the insertion procedure) to 1 (all the species in the

phylogenetic tree were inserted and were not presented in backbone phylogeny). PD_deficit

function returns a vector with three values, the Darwinian shortfall (Equation 1), the total

phylogenetic diversity calculated as the sum of branch lengths of the tree (PD_{total}) with all

species provided by the user, the sum of branch lengths inserted ($PD_{inserted}$) in the tree and

that was already present in the backbone tree ($PD_{present\ in\ tree}$). It is worth noting that the sum

of $PD_{inserted}$ and $PD_{present}$ are complementary, summing up to PD_{total}. To calculate the

Darwinian shortfall through the *PD_deficit* function, the user must provide a phylogenetic

tree and a table of insertions, both obtained from the *FishPhyloMaker* function.

Sensitivity analysis

We performed a sensitivity analysis to assess how the insertion procedure implemented

herein and the amount of inserted species affect two characteristics of phylogenetic trees: the

mean pairwise distance among species and the phylogenetic distinctiveness.

We 1) randomly change the name of a subsample of species within Rabosky's

phylogeny. Then, 2) we built a phylogeny for the species sampled with changed names in the

previous step using the FishPhyloMaker function. Finally, we computed: 3) the matrix

correlation (Pearson correlation) between the cophenetic distances of the subsampled species

in Rabosky's phylogeny and the FishPhyloMaker phylogeny; and 4) the Pearson correlation

between the phylogenetic distinctness values for the Rabosky's and FishPhyloMaker

phylogenies. The evolutionary distinctness was calculated as the equal splits measure that is

the sum of the contribution of all branches of a given lineage divided among its daughter

branches (Redding and Mooers, 2006). Evolutionary distinctness measure was calculated

using the phyloregion package (Daru et al., 2020).

The abovementioned steps (1, 2 and 3) were repeated 100 times for eleven different

quantities (10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%) of subsampled species

from Rabosky's phylogeny and inserted by the FishPhyloMaker function.

Illustrating the use of FishPhyloMaker package

We provide an example of the usage of the *FishPhyloMaker* package by creating a

phylogenetic tree using a global dataset of freshwater fishes inhabiting 3,119 freshwater

drainage basins that cover more than 80% of the Earth surface and 14886 species (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

built a phylogenetic for all species presented in Tedesco´s et al. dataset and mapped all the

insertions realized. Moreover, we used this same dataset to demonstrate how to map

Darwinian shortfalls, calculated following Equation 1 through *PD_deficit* function for all the

drainage basins in the Tedesco et al. (2017) dataset. All the analyses were performed using

the development version of the FishPhyloMaker package, which can be downloaded using

the following command line:

```
devtools::install_github("GabrielNakamura/FishPhyloMaker", ref = "main",
252    build_vignettes = TRUE)
```

253   We recommend that the user updates all the requested packages to avoid errors related to

254   packages versions. We first prepared the fish occurrence by checking the validity of its names

255   by using the function *FishTaxaMaker*. The occurrence matrix encompassed 14,886 species,

256   from which 13,992 were valid names. The remaining 961 names were substituted by their

257   corresponding valid names according to FishBase. We applied the *FishPhyloMaker* function

258   to build a phylogenetic tree containing all the 14,886 species with valid names retrieved from

259   *FishTaxaMaker* (Figure 2). For simplicity and reproducibility, we set the argument

260   `insert.base.node` as TRUE, thus, inserting all species at the base node of its corresponding

261   family and order when needed. We also set the argument `return.insertions = TRUE` for

262   retrieving the insertion information of each species. Then, we applied the *PD_deficit* function

263   to calculate the Darwinian shortfall for all the freshwater basins of the world harboring at

264   least two species (Tedesco et al. 2017). The *PD_deficit* function was calculated considering

265   congeneric insertions and insertions at the family level, however, the function may also

266   include other levels of phylogenetic insertion, like order insertions. All the codes need to

267   fully reproduce these analyses are provided at the GitHub repository

268   (GabrielNakamura/MS_FishPhyloMaker). For further explanations and examples illustrating

269   the usage of functions in the FishPhyloMaker package, the user can assess the package

270   website https://gabrielnakamura.github.io/FishPhyloMaker/index.html and see the Articles

271   section.

272   **Results**

273   The entire insertion procedure lasted approximately three hours using one core from a

274   computer machine with an i5 processor. A total of 11,569 species were inserted, 6,418

275   species were already present in the backbone phylogeny, and 181 were not inserted at all,

276   resulting in a phylogenetic tree containing 14,705 species (Figure 2). We also showed in

277   Figure 2 all the insertions realized through the FishPhyloMaker function and the seven orders

278   of ray-finned fishes with that present the highest number of species. We can see in Figure two

279   that the insertions are evenly distributed throughout the phylogenetic tree.

281   Figure 2: Phylogenetic tree obtained from FishPhyloMaker, containing 14,705 finned-ray

282   species with their respective insertions. We also highlight in the gray rectangles the seven

283   most speciose Orders.

285   We also depicted all the insertions made by FishPhyloMaker for all freshwater

286   Ecoregion of the world. This was only possible because FishPhyloMaker flags all the

287   insertions made during the insertion procedure. Figure 3 shows that Neotropics and

288   Afrotropics regions exhibited the largest number of species inserted. On the contrary, despite

289   the great area and number of basins, the Nearctic Ecoregion presented the smallest percentage

290   of insertions, most of them congeneric. All Ecoregions and the percentage of species

291   insertions per level are shown in Figure 3.

Figure 3: Barplot showing the percentage of species inserted in each one of the seven freshwater ecoregions of the world and their respective type of insertions mapped by FishPhyloMaker package.

We spatialized the Darwinian shortfalls per basin and observed that tropical regions exhibited the highest shortfalls, while northern sites had the lowest (Figure 4). The highest values of Darwinian shortfalls were found in Afrotropics and Neotropics, as some drainages did not harbor any (or only a few) species in the Rabosky's phylogeny. The grey areas correspond to sites that do not present species occurrences accordingly to Tedesco et al. (2017) or presented less than two occurrences for the Order considered. We also depicted the

302   Darwinian shortfalls for the four major orders in terms of species richness (bottom maps in

303   Figure 4). For all the groups, the highest values of Darwinian shortfalls were found in the

304   neotropical region, except for Cypriniformes, the group responsible for the highest values of

305   Darwinian shortfalls in the watersheds in Asia and some basins in North America.

308   Figure 4: Global distribution of the Darwinian Shortfalls for ray-finned fishes, based on

309   freshwater species occurrences in more than 3000 basins. Values near to 1 indicate a high

310   Darwinian shortfall (a large number of congeneric insertions), while values near zero indicate

311   low shortfalls. We depicted the Darwinian shortfall for the four major orders in terms of

312   species richness (Characiformes, Cypriniformes, Cichliformes, and Siluriformes). Gray color

313   indicates areas with no occurrence of species for a given order.

315   The sensitivity analysis highlights the strong correlation between the cophenetic distances of

316   Rabosky's and FishPhyloMaker phylogenies (Figure 5 B) even in varying levels of taxa

317   insertions. Inversely, an increasing number of insertions on Rabosky's phylogeny reduced the

318   correlation between phylogenetic distinctness in the original phylogeny and that assembled

319   by FishPhyloMaker (Figure 5 A).

Figure 5: Barplots showing the correlation of evolutionary distinctness values (A) and

between cophenetic distances (B) calculated from original phylogeny and inserted

phylogenies with varying percentages of species inserted in the original phylogeny. Grey dots

325 represent individual correlation values. The lower and upper hinges in boxplots represent the

326 first and third quantiles while the middle hinge represents the median.

Discussion

328 We provided a user-friendly, fast, reliable, and reproducible way to construct phylogenetic

329 trees for a megadiverse group (Actinopterygii). The FishPhyloMaker package is in line with

330 tools developed for plants, such as Phylomatic (C++ application) and V.PhyloMaker (R

331 package) (Jin and Qian, 2019; Webb and Donoghue, 2005), but includes different features.

332 These features include new options for inserting species through an interactive procedure in

333 phylogenies and recording insertions. The latter feature allows a better systematization of

334 building supertrees and calculating the first, to our knowledge, quantitative measure of the

335 Darwinian shortfall.

336 Whereas Phylomatic allows the insertion of absent species only as congeneric or at

337 the node corresponding to the family of the focal species (Webb and Donoghue, 2005), the

338 FishPhyloMaker package delivers options through an interactive procedure of insertion. The

339 performed insertions can be easily recorded in an R script, providing flexibility and the same

340 level of reproducibility as other algorithms designed for similar purposes (*e.g.*, Jin and Qian,

341 2019). This interactive option is a novelty when compared to similar insertion algorithms

342 (*e.g.*, Phylomatic).

343 The spatial distribution of the Darwinian shortfall is paramount to guide our future

344 efforts to understand the history of life. The phylogenetic gaps in the knowledge of ray-

345 finned fishes are geographically biased, with tropical basins presenting higher Darwinian

346 shortfalls levels, as evidenced in this study. This gap in evolutionary knowledge could lead to

347 a bias in evaluating the effects of evolutionary history and the interpretation of

348 macroecological patterns for fish assemblages in these regions, which can affect conservation

349 decisions based on the phylogenetic dimension of diversity (Assis, 2018).

350   Several biological and sociological factors can explain the observed bias in Darwinian

351   shortfalls. First, the regions exhibiting the most significant Darwinian gaps also exhibit the

352   largest freshwater fish diversity, which we can not describe at the same speed as less

353   biologically rich areas (Hortal et al., 2015). Second, on-ground accessibility, human

354   occupation, and economic development constrain investments in biodiversity research

355   (Moura et al., 2018; Moura and Jetz, 2021), which is probably more pronounced in tropical

356   regions than temperate ones, which may hamper field sampling and phylogenetic analyses.

357   Despite being more simple when compared with other insertion methods (e.g., Pearse

358   and Purvis, 2013), FishPhyloMaker provided reliable results by preserving important

359   characteristics of the phylogenetic tree, as we showed through the sensitivity analysis.

360   Commonly used measures of phylogenetic diversity are based on the pairwise distance of

361   species from a phylogenetic tree (e.g., Kraft et al., 2007; Webb et al., 2002), and we showed

362   that the algorithm implemented in FishPhyloMaker successfully preserve the distances

363   among species in the phylogenetic tree even for a great number of insertions.

365   *Limitations and possible applications*

366   Future developments of the package should consider the Catalog of Fishes (van der

367   Laan et al., 2021) to improve the nomenclature checking procedures. Despite Fishbase being

368   a widely used database to check for the taxonomic classification of fishes, it may present

369   delays in updating taxonomic information because it is not its primary purpose. Inversely, the

370   Catalog of Fishes is an authoritative taxonomic list frequently updated.

371   An inherent limitation of the phylogenetic hypothesis produced by FishPhyloMaker is

372   the large number of polytomies resulting from the insertion procedures. We recommend that

373   users directly assess how the phylogenetic uncertainty affects further analysis when not using

374   a fully solved phylogenetic tree (Martins et al., 2013). Furthermore, we recommend caution

375   in the use of FishPhyloMaker phylogenies to compute measures that depend on speciation

376   events (e.g., evolutionary distinctiveness and other split-based metrics) since the insertion

377   procedure modifies the split events in the tree as shown in the sensitivity analysis.

378   These limitations do not preclude the package applicability for studies in phylogenetic

379   community ecology since synthesis phylogenies do not significantly impact phylogenetic

380   diversity indices as showed by previous studies (Li et al., 2019) and confirmed in ours

381   (through sensitivity analysis). Moreover, this is the only automated tool able to provide a

382   complete phylogenetic tree that can easily handle large datasets. FishPhyloMaker can be

383   relevant for addressing several critical questions in ecology and evolution by facilitating the

384   obtention of phylogenetic hypotheses for local pools of ray-finned fishes. This facilitation can

385   be essential for regions with a large gap in the phylogenetic knowledge of fishes, such as the

386   Neotropical region (Albert et al., 2020). Such phylogenetic hypotheses allow understanding

387   how ecological traits evolved or how the current and past environmental conditions selected

388   the lineages in different areas.

389   Biogeographical studies are usually restricted to one or a few lineages at larger scales

390   due to the availability of molecular phylogenies (e.g. García-Andrade et al., 2021) or with

391   phylogenies with a considerable number of absent species (Miller, 2021). The

392   FishPhyloMaker package facilitates large-scale investigations on the biogeographic history of

393   the most diverse group of vertebrates on Earth, the Actinopterygians, helping us understand

394   the processes that drive this high diversity. Finally, we can map where the lack of

395   phylogenetic information is the most critical once the function returns the insertion level of

396   species. This information can directly elucidate the patterns of the Darwinian shortfalls for

397   ray-finned fishes, contributing not only to direct sampling and studying efforts but also to

398   evidence the need for increased efforts to decolonize science (Trisos et al., 2021). Therefore,

399   we expect that the FishPhyloMaker package reduces the gaps and barriers to addressing

400   ecological and evolutionary questions due to the difficulty or lack of a reliable phylogenetic

401   hypothesis for local and regional pools of ray-finned fishes.

403   **Contributions**

404   GN Conceptualization; Data curation; Formal Analysis; Methodology; Software; Writing –

405   original draft. AR Data curation; Methodology; Software, Writing – review, and editing. BES

406   Writing – original draft; Methodology.

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