1   DateLife: leveraging databases and analytical tools to reveal the dated Tree of Life

2   Luna L. Sánchez Reyes[1,2], Emily Jane McTavish[1], & Brian O'Meara[2]

3   [1] University of California, Merced, USA

4   [2] University of Tennessee, Knoxville, USA

5   Author Note

6 Department of Life and Environmental Sciences, University of California, Merced, CA

7 95343, USA.

8 Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville,

9 446 Hesler Biology Building, Knoxville, TN 37996, USA.

15 Correspondence concerning this article should be addressed to Luna L. Sánchez Reyes, .

16 E-mail: sanchez.reyes.luna@gmail.com

DateLife: leveraging databases and analytical tools to reveal the dated Tree of Life

Abstract

Chronograms –phylogenies with branch lengths proportional to time– represent key data on timing of evolutionary events for the study of natural processes in many areas of biological research. Chronograms also provide valuable information that can be used for education, science communication, and conservation policy decisions. Yet, achieving a high-quality reconstruction of a chronogram is a difficult and resource-consuming task. Here we present DateLife, a ~~service~~ phylogenetic software implemented as an R package and an R Shiny web application available at www.datelife.org, that provides services for efficient and easy discovery, summary, reuse, and reanalysis of node age data mined from a curated database of expert, peer-reviewed, and openly available chronograms. The main DateLife workflow starts with one or more scientific taxon names provided by a user. Names are processed and standardized to a unified taxonomy, allowing DateLife to run a name match across its local chronogram database that is curated from Open Tree of Life's phylogenetic repository, and extract all chronograms that contain at least two queried taxon names, along with their metadata. Finally, node ages from matching chronograms are mapped using the congruification algorithm to corresponding nodes ~~from a chosen tree topologyusing the congruification algorithm~~on a tree topology, either extracted from Open Tree of Life's synthetic phylogeny or one provided by the user. Congruified node ages are used as secondary calibrations to date the chosen topology, with or without initial branch lengths, using different phylogenetic dating methods such as BLADJ, treePL, PATHd8 and MrBayes. We performed a cross-validation test to compare node ages resulting from a DateLife analysis (i.e, phylogenetic dating using secondary calibrations) to those from the original chronograms (i.e, obtained with primary calibrations), and found that DateLife's node age estimates are consistent with the age estimates from the original chronograms, with the largest variation in ages occurring around topologically deeper nodes. ~~Results~~ Because the results from any

software for scientific analysis can only be as good as the data used as input, we highlight the importance of considering the results of a DateLife analysis in the context of the input chronograms. ~~We encourage the use of DateLife to help~~ DateLife can help to increase awareness of the existing disparities among alternative hypotheses of dates for the same diversification events, and to support exploration of the effect of alternative chronogram hypotheses on downstream analyses, providing a framework for a more informed interpretation of evolutionary results.

Keywords: Tree; Phylogeny; Scaling; Dating; Ages; Divergence times; Open Science; Congruification; Supertree; Calibrations; Secondary calibrations.

Word count: ~~6707~~ 7042

Chronograms –phylogenies with branch lengths proportional to time– provide key data on evolutionary time frame for the study of natural processes in many areas of biological research, such as comparative analysis (Freckleton, Harvey, & Pagel, 2002; Harvey, Pagel, & others, 1991), developmental biology (Delsuc et al., 2018; Laubichler & Maienschein, 2009), conservation biology and ecology (Felsenstein, 1985; Webb, 2000), historical biogeography (Posadas, Crisci, & Katinas, 2006), and species diversification (Magallon & Sanderson, 2001; Morlon, 2014).

Building a chronogram is not an easy task. It requires obtaining and curating a homology hypothesis to construct a phylogeny, selecting and placing appropriate calibrations on the phylogeny using independent age data points from the fossil record or other dated events, and inferring a full dated tree. All of this entails specialized biological training, taxonomic domain knowledge, and a significant amount of research time, computational resources and funding.

Here we present the DateLife project which has the main goal of extracting and exposing age data from published chronograms, making age data readily accessible to a wider community for reuse and reanalysis in research, teaching, science communication and conservation policy. DateLife's core software application is available as an R package (Sanchez-Reyes et al., 2022), and as an online Rshiny interactive website at www.datelife.org. It features key elements for scientific reproducibility, such as a curated, versioned, open and fully public chronogram database (McTavish et al., 2015) that stores data in a computer-readable format (Vos et al., 2012); automated and programmatic ways of accessing and downloading the data, also in a computer-readable format (Stoltzfus et al., 2013); and methods to summarize and compare the data.

DESCRIPTION

DateLife's core software applications are implemented in the R package `datelife`, and

relies on functionalities from other biological R packages: ape (Paradis, Claude, & Strimmer, 2004), bold (Chamberlain, 2018), geiger (Pennell et al., 2014), msa (Bodenhofer, Bonatesta, Horejš-Kainrath, & Hochreiter, 2015), paleotree (Bapst, 2012), phyloch (Heibl, 2008), phylocomr (Ooms & Chamberlain, 2018), phytools (Revell, 2012), rotl (Michonneau, Brown, & Winter, 2016), and taxize (Chamberlain, 2018; Chamberlain & Szöcs, 2013). ~~Figure 1 provides a graphical summary of the~~ There are three main steps ~~of~~ to the DateLife workflow: 1) creating a search query, 2) searching a database, and 3) summarizing results from the search.

Creating a Search Query

DateLife starts by processing an input consisting of the scientific name of at least one taxon. Multiple input names can be provided as a comma separated character string or as tip labels on a tree. If the input is a tree, it can be provided as a classic newick character string (Archie et al., 1986), or as a "phylo" R object (Paradis et al., 2004). The input tree is not required to have branch lengths, and its topology is used in the summary steps described in the next section.

DateLife processes input scientific names using a Taxonomic Name Resolution Service (TNRS), which increases the probability of correctly finding the queried taxon names in the chronogram database. TNRS detects, corrects and standardizes name misspellings and typos, variant spellings and authorities, and nomenclatural synonyms to a single taxonomic standard (Boyle et al., 2013). TNRS also allows to correctly choose between homonyms, by considering other taxa provided as input to infer the taxonomic context of the homonym. DateLife implements TNRS using the Open Tree of Life (OpenTree) unified Taxonomy (OTT, Open Tree Of Life et al., 2016; Rees & Cranston, 2017) as standard, storing taxonomic identification numbers (OTT ids) for further processing and analysis. Other taxonomies currently supported by DateLife are the National Center of Biotechnology Information (NCBI) taxonomic database (Schoch et al., 2020), the Global Biodiversity

104 Information Facility (GBIF) taxonomic backbone (GBIF Secretariat, 2022), and the Interim

105 Register of Marine and Non-marine Genera (IRMNG) database (Rees et al., 2017).

106 Besides binomial species names, DateLife accepts scientific names from any inclusive

107 taxonomic group (e.g., genus, family, tribe), as well as subspecific taxonomic variants (e.g.,

108 subspecies, variants, strains). If a taxon name belongs to an inclusive taxonomic group,

109 DateLife has two alternative behaviors defined by the "get species from taxon" flag. If the

110 flag is active, DateLife retrieves all species names within a taxonomic group provided, from a

111 standard taxonomy of choice, and adds them to the search query. In this case, subspecific

112 variants are excluded. If the flag is inactive, DateLife excludes inclusive taxon names from

113 the search query, and species and subspecific variant names are processed as provided by the

114 user. The processed taxon names are saved as an R object of a newly defined class,

115 `datelifeQuery`, that is used in the following steps. This object contains the input names

116 standardized to a taxonomy of choice (OTT by default), the corresponding OTT id numbers,

117 and the topology of an input tree, if one was provided.

Searching a Chronogram Database

119 At the time of writing of this manuscript (~~Jun 11~~Nov 14, 2023), DateLife's chronogram

120 database latest version consist of 253 chronograms published in 187 different studies,

121 encompassing close to 100 k (99 474) different species. It is curated from OpenTree's

122 phylogenetic database, the Phylesystem, ~~which constitutes an open source~~ an open database

123 of expert and peer-reviewed phylogenetic knowledge with rich metadata and a wide

124 taxonomic scope (McTavish et al., 2015)~~, which allows automatic and reproducible~~

125 ~~assembly of our chronogram database. Datelife's chronogram database is navigable as an R~~

126 ~~data object within the `datelife` R package.~~ We expect DateLife's database to largely

127 overlap with OpenTree's phylogenetic database taxonomic coverage, where Chordata and

128 Embryophyta are nearly fully sampled. In contrast, Bacteria, Fungi, Nematoda, and

129 Insecta, currently present a large gap between the number of named species and what has

¹³⁰ phylogenetic information in OpenTree's synthetic tree. It is likely that users working with

¹³¹ the former groups will get results from a DateLife analysis. If none of the user's species are

¹³² found, the software will indicate the lack of age data for the queried taxa in the database.

¹³³ A unique feature of the Phylesystem is that any user can add new published,

¹³⁴ state-of-the-art chronograms any time, through OpenTree's curator application

¹³⁵ (https://tree.opentreeoflife.org/curator). Relying on an open source database permits an

¹³⁶ automatic and reproducible assembly of DateLife's chronogram database, which is stored

¹³⁷ and navigable as an R data object within the `datelife` R package. As chronograms are

¹³⁸ added to Phylesystem, they can be incorporated into the chronogram database ~~of~~ within the

¹³⁹ `datelife` R package, ~~which is currently manually updated as new chronogram data is~~

¹⁴⁰ ~~added to Phylesystem~~by manually triggering an update. The updated `datelife` database

¹⁴¹ is assigned a new version number, followed by a package release on CRAN. We encourage

¹⁴² users to submit published chronograms to OpenTree's phylogenetic database, so that their

¹⁴³ taxon of interest can be included in future DateLife searches. Users can directly run

¹⁴⁴ `datelife` functions to trigger an update of their local chronogram database, to incorporate

¹⁴⁵ any new chronograms to their DateLife analysis before ~~an official~~ a `datelife` database

¹⁴⁶ update is released on CRAN.

¹⁴⁷ A DateLife search is implemented by matching processed taxon names provided by the

¹⁴⁸ user to tip labels in the chronogram database. Chronograms with at least two matching

¹⁴⁹ taxon names on their tip labels are identified and pruned down to preserve only the matched

¹⁵⁰ taxa. These matching pruned chronograms are referred to as source chronograms. Total

¹⁵¹ distance in units of million years (Myr) between taxon pairs within each source chronogram

¹⁵² are stored as a patristic distance matrix~~(Fig. 1).~~. The matrix format speeds up extraction

¹⁵³ of pairwise taxon ages of any queried taxa, as opposed to searching the ancestor node of a

¹⁵⁴ pair of taxa in a "phylo" object or newick string. Finally, the patristic matrices are

¹⁵⁵ associated to the study citation where the original chronogram was published, and stored as

156 an R object of the newly defined class `datelifeResult`.

157   *Summarizing Search Results*

158   Summary information is extracted from the `datelifeResult` object to inform

159 decisions for subsequent steps in the analysis workflow. Basic summary information available

160 to the user ~~is~~includes:

161   1. The matching pruned chronograms as newick strings or "phylo" objects.

162   2. The ages of the root of all source chronograms. These ages can correspond to the age

163   of the most recent common ancestor (mrca) of the user's group of interest if the source

164   chronograms have all taxa belonging to the group. If not, the root corresponds to the

165   mrca of a subgroup within the group of interest.

166   3. Study citations where original chronograms were published.

167   4. A report of input taxon names matches across source chronograms.

168   5. The source chronogram(s) with the most input taxon names.

169   6. Various single summary chronograms resulting from summarizing age data, generated

170   using the methodology described next.

171   *Choosing a Topology*

172   DateLife requires a tree topology to summarize age data upon. We recommend that

173 users provide as input a tree topology from the literature, or one of their own making. If no

174 topology is provided, DateLife automatically extracts one from the OpenTree synthetic tree,

175 a phylogeny currently encompassing 2.3 million taxa across all life, assembled from 1,239

176 published phylogenetic trees and OpenTree's unified Taxonomy, OTT (Open Tree Of Life et

177 al., 2019). Alternatively, DateLife can combine topologies from source chronograms using a

178 supertree approach (Criscuolo, Berry, Douzery, & Gascuel, 2006). To do this, DateLife first

179 identifies the source chronograms that form a grove, roughly, a sufficiently overlapping set of

180 taxa between trees, by implementing definition 2.8 for n-overlap from Ané et al. (2009). If

181 the source chronograms do not form a grove, the supertree reconstruction will fail. In rare

182 cases, a group of trees can have multiple groves. By default, DateLife chooses the grove with

183 the most taxa, however, the "criterion = trees" flag allows the user to choose the grove with

184 the most trees instead. The result is a single summary (~~or~~ i.e., supertree) topology, that

185 combines topologies from source chronograms in a grove.

Applying Secondary Calibrations

187 Once a topology is chosen, DateLife applies the congruification method (Eastman,

188 Harmon, & Tank, 2013) that find nodes belonging to the same clade across source

189 chronograms, and then extracts the corresponding node ages from patristic distance matrices

190 stored as a `datelifeResult` object. Note that by definition, these matrices store total

191 distance (time from tip to tip), assuming that the terminal taxa are coeval and occur at the

192 present. Hence, node ages correspond to half the values stored in the `datelifeResult`

193 matrices. A table of congruified node ages that can be used as calibrations for a dating

194 analysis is stored as a `congruifiedCalibrations` object.

195 For each congruent node, the pairwise distances that traverse that node are summarized

196 into a single summary matrix using classic summary statistics (i.e., mean, median, minimum

197 and maximum ages), and the Supermatrix Distance Method (SDM; Criscuolo et al., 2006),

198 which deforms patristic distance matrices by minimizing variance and then averaging them.

199 These single summary taxon pair age matrices are stored as summarized calibrations that

200 can be used as secondary calibrations to date a tree topology - with or without initial branch

201 lengths, using phylogenetic dating methods currently supported within DateLife: BLADJ

202 (Webb, Ackerly, & Kembel, 2008; Webb & Donoghue, 2005), MrBayes (Huelsenbeck &

203 Ronquist, 2001; Ronquist & Huelsenbeck, 2003), PATHd8 (Britton, Anderson, Jacquet,

204 Lundqvist, & Bremer, 2007), and treePL (Smith & O'Meara, 2012).

Dating a Tree Topology

Dating a tree without branch lengths. – ~~When producing or obtaining a tree with branch lengths for a group of interest is not possible, DateLife can date a topology without branch lengths , obtained from OpenTree or by implementing the supertree approach described above, by applying the~~ To date a tree topology when initial branch lengths are unavailable, DateLife implements the Branch Length Adjuster (BLADJ~~;~~) algorithm (Webb et al., 2008; Webb & Donoghue, 2005)~~algorithm, which requires no initial branch lengths . The algorithm starts by fixing~~ , which only requires a tree topology with no branch lengths and at least two node ages to use as calibrations, one for the tree root and one for any internal node of the topology. The BLADJ algorithm fixes ages for nodes with calibration data upon the given tree topology. Then, it ~~distributes time for~~ assigns ages to nodes with no ~~data~~ available age information by distributing time evenly between calibrated nodes, minimizing age variance in the resulting chronogram. This approach has proven useful for ecological analyses that require a phylogenetic time context (Webb et al., 2008). When there is conflict between ages of calibrated nodes, BLADJ ignores node ages that are older than the age of a parent node. The BLADJ algorithm requires a root age ~~estimate~~ to run. ~~If~~ Users can provide an appropriate root age estimate of their own or one obtained from the literature. If a root age is not provided and there is no information on the age of the root in the chronogram database, ~~users can provide an estimate from the literature. If none is provided, DateLife will choose an~~ DateLife chooses a random age for the root~~so that it can return a dated topology. It will also~~ , so that a dated tree topology can be generated with BLADJ. In this case, DateLife will provide a conspicuous warning message, so that users are aware that the root of the chronogram ~~does not have information available~~ was chosen at random because there was no information available for it in the chronogram database, along with suggestions on how the user can find and provide an appropriate age for the root of the initial topology.

~~In the absence of genetic data, BLADJ is a very agnostic way to assign ages to nodes with no available data, as it does not require any assumptions on the underlying model of~~

~~branch length distribution. It is however~~ An alternative to BLADJ to date tree topologies in the absence of initial branch lengths that is common practice in the literature is to use a birth-death model to ~~assign ages to nodes with no genetic data~~ draw branch lengths (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012; Rabosky et al., 2018; Smith & Brown, 2018). ~~To do so, DateLife implements~~ In addition to the initial tree topology and nodes with age data, these methods require initial values of speciation and extinction rate parameters provided by the user. DateLife implements this approach with MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), using nodes with published age data as calibration priors on ~~a fixed topology~~ nodes of a tree topology with no branch lengths, a simple birth-death model with ~~parameters that can be determined~~ speciation and extinction rate parameters that are provided by the user, and no genetic data. However, BLADJ is the default option in DateLife, as it does not require any information on diversification rates for the phylogenetic sample to draw from a branch length distribution.

Dating a tree with branch lengths.– Relative branch lengths can provide key information for phylogenetic dating, specifically for nodes without any calibration data available. While using initial branch length data is the golden standard for phylogenetic dating analyses, ~~estimating trees with branch lengths proportional to substitution rates per site~~ obtaining such information from scratch is not an easy task: it requires obtaining primary data, assembling and curating a homology (orthology) hypothesis, and choosing and implementing a method for phylogenetic inference. DateLife implements a workflow to streamline this process by applying open data from the Barcode of Life Data System, BOLD (Ratnasingham & Hebert, 2007) to obtain genetic markers for input taxa. By default, BOLD genetic sequences are aligned with MUSCLE (Edgar, 2004) using functions from the msa R package (Bodenhofer et al., 2015). Alternatively, sequences can be aligned with MAFFT (Katoh, Asimenos, & Toh, 2009), using functions from the ape R package (Paradis et al., 2004). The BOLD sequence alignment is then used to obtain initial branch lengths with the accelerated transformation (ACCTRAN) parsimony algorithm, which resolves ambiguous

²⁶⁰ character optimization by assigning changes along branches of the tree as close to the root as

²⁶¹ possible (Agnarsson & Miller, 2008), resulting in older internal nodes as compared to other

²⁶² parsimony algorithms (Forest et al., 2005). The parsimony branch lengths are then

²⁶³ optimized using Maximum Likelihood, given the alignment, the topology and a simple

²⁶⁴ Jukes-Cantor model, producing a BOLD tree with branch lengths proportional to expected

²⁶⁵ number of substitutions per site. Both parsimony and ML optimizations are done with

²⁶⁶ functions from the `phangorn` package (Schliep, 2011). Due to the computing load it requires,

²⁶⁷ the BOLD workflow is currently only supported through DateLife's R package. It is not yet

²⁶⁸ available through the web application.

²⁶⁹   Phylogenetic dating methods supported in DateLife that incorporate branch length

²⁷⁰ information from the input topology in combination with the secondary calibrations include:

²⁷¹ PATHd8, a non-clock, rate-smoothing method to date trees (Britton et al., 2007); treePL

²⁷² (Smith & O'Meara, 2012), a semi-parametric, rate-smoothing, penalized likelihood dating

²⁷³ method (Sanderson, 2002); and MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist &

²⁷⁴ Huelsenbeck, 2003), a Bayesian inference program implementing Markov chain Monte Carlo

²⁷⁵ (MCMC) methods to estimate a posterior distribution of model parameters.

²⁷⁶   *Visualizing Results*

²⁷⁷   Finally, users can save all source and summary chronograms in formats allowing for

²⁷⁸ reuse and reanalysis, such as newick and the R "phylo" format. Input and summary

²⁷⁹ chronograms can be visualized and compared graphically, and users can construct their own

²⁸⁰ graphs using DateLife's chronogram plot generation functions available from the R package

²⁸¹ `datelifeplot` (Sanchez-Reyes & O'Meara, 2022).

²⁸²   BENCHMARK

²⁸³   R package `datelife` code speed was tested on an Apple iMac with one 3.4 GHz Intel

²⁸⁴ Core i5 processor. We registered variation in computing time of query processing and search

through the database relative to number of queried taxon names. Query processing time

increases roughly linearly with number of input taxon names, and increases considerably if

Taxonomic Name Resolution Service (TNRS) is activated. Up to ten thousand names can be

processed and searched in less than 30 minutes with the most time consuming settings. Once

names have been processed as described in methods, a name search through the chronogram

database can be performed in less than a minute, even with a very large number of taxon

names (Fig. ~~2~~1).

`datelife`'s code performance was evaluated with a set of unit tests designed and

implemented with the R package testthat (R Core Team, 2018) that were run both locally

with the devtools package (R Core Team, 2018), and on a public server using the continuous

integration tool of GitHub actions (https://docs.github.com/en/actions). At present, unit

tests cover more than 40% of `datelife`'s code (https://codecov.io/gh/phylotastic/datelife).

Unit testing helps identify potential issues as code is updated or, more critically, as services

code relies upon may change.

<div align="center">CASE STUDIES</div>

We illustrate the DateLife workflow using a family within the passeriform birds

encompassing the true finches, Fringillidae, as case study. On a small example, we analysed 6

bird species, and results from each step of the workflow are shown in Figure ~~3.~~ 2. As a

second example, we analysed 289 bird species in the family Fringillidae that are included in

the NCBI taxonomy. ~~The summary chronogram resulting~~ One clade from the full summary

chronogram result from the DateLife analysis is shown ~~in Figure 5, and~~ Figure 3. The full

chronogram for all 289 species and the results from previous steps of the workflow are

available as Supplementary Figures.

<div align="center">*A Small Example*</div>

Creating a search query.– We chose 6 bird species within the Passeriformes. The

sample includes two species of cardinals: the black-thighed grosbeak – *Pheucticus tibialis* and the crimson-collared grosbeak – *Rhodothraupis celaeno*; three species of buntings: the yellowhammer – *Emberiza citrinella*, the pine bunting – *Emberiza leucocephalos* and the yellow-throated bunting – *Emberiza elegans*; and one species of tanager, the vegetarian finch – *Platyspiza crassirostris*. Processing of input names found that *Emberiza elegans* is synonym for *Schoeniclus elegans* in the default reference taxonomy (OTT v3.3, June 1, 2021). For a detailed discussion on the state of the synonym, refer to Avibase (Avibase, 2022; Lepage, 2004; Lepage, Vaidya, & Guralnick, 2014). Discovering this synonym allowed assigning five age data points for the parent node of *Emberiza elegans*, shown as *Schoeniclus elegans* in Figure ~~3a~~2, which would not have had any data otherwise.

Searching the database. – DateLife used the processed input names to search the local chronogram database and found 9 matching chronograms from 6 different studies (Fig. ~~3b~~2c). Three studies matched five input names (Barker, Burns, Klicka, Lanyon, & Lovette, 2015; Hedges, Marin, Suleski, Paymer, & Kumar, 2015; Jetz et al., 2012), one study matched four input names (Hooper & Price, 2017) and two studies matched two input names (Barker, Burns, Klicka, Lanyon, & Lovette, 2013; Burns et al., 2014). No studies matched all input names. Together, source chronograms provide 28 unique age data points, covering all nodes on our chosen tree topology to date (Table 1).

Summarizing search results. – DateLife obtained OpenTree's synthetic tree topology for these taxa (Fig. ~~3c~~2d), and congruified and mapped age data to nodes in this chosen topology, shown in Table 1. The name processing step allowed including five data points for node "n4" (parent of *Schoeniclus elegans*~~; Fig. 3A~~) that would not have had any data otherwise due to name mismatch. Age summary statistics per node were calculated (Table 2) and used as calibrations to date the tree topology using the BLADJ algorithm. As expected, more inclusive nodes (e.g., node "n1") have more variance in age data than less inclusive nodes (e.g., node "n5"). Median summary age data for node "n2" was excluded as

336 final calibration because it is older than the median age of a more inclusive node, "n1" (Fig.

337 3c42g).

An Example with the Family of True Finches

339   ***Creating a query.*** – To obtain ages for all species within the family of true finches,

340 Fringillidae, we ran a DateLife query using the "get species from taxon" flag, which gets all

341 recognized species names within a named group from a taxonomy of choice. Following the

342 NCBI taxonomy, our DateLife query has 289 Fringillidae species names. This

343 taxon-constrained approach implies that the full DateLife analysis will be performed using a

344 tree topology and ages available for species names from a given taxonomic group, which do

345 not necessarily correspond to a monophyletic group. Users can change this behavior by

346 providing all species names corresponding to a monophyletic group as input for a DateLife

347 search, or a monophyletic tree to construct a DateLife summary.

348   ***Searching the database.*** – Next, we used the processed species names in our

349 DateLife query to identify chronograms with at least two Fringillidae species as tip taxa.

350 The DateLife search identified 19 chronograms matching this criteria, published in 13

351 different studies (Barker et al., 2013, 2015; Burns et al., 2014; Claramunt & Cracraft, 2015;

352 Gibb et al., 2015; Hedges et al., 2015; Hooper & Price, 2017; Jetz et al., 2012; Kimball et al.,

353 2019; Oliveros et al., 2019; Price et al., 2014; Roquet, Lavergne, & Thuiller, 2014; Uyeda,

354 Pennell, Miller, Maia, & McClain, 2017). Once identified, DateLife pruned these matching

355 chronograms to remove tips that do not belong to the queried taxon names, and transformed

356 these pruned chronograms to pairwise distance matrices, revealing 1,206 different age data

357 points available for species within the Fringillidae (Supplementary Table S1).

358   ***Summarizing search results.*** – The final step entailed congruifying and

359 summarizing the age data available for the Fringillidae species into two single summary

360 chronograms, using two different types of summary ages, median and SDM. As explained in

361 the "Description" section, a tree topology to summarize age data upon is required. By

362 default, DateLife uses the topology from OpenTree's synthetic tree that contains all taxa

363 from the search query. According to OpenTree's synthetic tree, species belonging to the

family Fringillidae do not form a monophyletic group (~~Fig. 4a~~Supplementary Fig. S1).

Hence, a topology containing only the 289 species from the original query was extracted from

Open Tree of Life's synthetic tree v12.3 (~~Fig. 4b~~Supplementary Fig. S2; Open Tree Of Life

et al., 2019).

~~Source~~ All 19 source chronograms (Supplementary Figs. ~~S2-S20~~S5-S23) were

congruified to OpenTree's topology shown in ~~Figure 4b~~Supplementary Figure S2, reducing

the original 1,206 node age data set to 818 different data points (Supplementary Table S2)

that ~~can~~ could be used as calibrations for the chosen topology (~~Fig. 4b~~Supplementary Fig.

S2). The congruent node age data points were summarized for each node, resulting in 194

summary node ages. From these 21 were excluded as secondary calibrations because they

were older than the ancestral node. The remaining 173 summary node ages were used as

secondary calibrations to obtain a fully dated (and resolved) phylogeny with the program

BLADJ (~~Fig. 5).~~ Supplementary Figure S3). Results for a subgroup are shown in Figure 3.

Cross-Validation Test

We performed a cross validation test of a DateLife analysis using the Fringillidae

source chronograms obtained above (Supplementary Figs. ~~S2-S20). We used as~~ S5-S23). As

inputs for a DateLife analysis, we used all individual tree topologies from each of the 19

source chronograms from 13 studies, treating their node ages as unknown. We congruified

node ages extracted from chronograms from all other studies upon the individual topologies,

effectively excluding original ages from each topology. Finally, average node ages per node

were applied as secondary calibrations and smoothed with the BLADJ algorithm. We found

that node ages from the original studies, and ages estimated using all other age data

available are ~~largely~~ generally correlated (Fig. ~~6~~4). For five studies, DateLife tended to

underestimate ages for topologically deeper nodes (those with many descendant taxa, aka

"closer to the root") relative to the original estimate, and overestimate ages for nodes closer

to the tips. Accordingly, root ages are generally older in the original study than estimated

using cross-validated ages (Supplementary Fig. ~~S1~~S4). In general, topologically deeper nodes display the largest age variation between node ages from the original chronograms and ages summarized with DateLife.

Discussion

DateLife's goal is to improve availability~~and accessibility~~, accessibility, and reusability of state-of-the-art data on evolutionary time frame of organisms, to allow users from all areas of science and with all levels of expertise to compare, ~~reuse,~~ use and reanalyse expert age data for their own applications. As such, it is designed as an open service that does not require any expert biological knowledge –besides the scientific names of the species or group that users want to work ~~with,~~ with– to use any of its functionalities.

A total of 99,474 unique terminal taxa are represented in DateLife's database. Incorporation of more chronograms into the database will continue to improve DateLife's services. One option to increase the number of chronograms in the DateLife database is the Dryad data repository. Methods to automatically mine chronograms from Dryad could be designed and implemented. However, Dryad's metadata system has no information to automatically detect branch length units, and those would still need to be determined manually by a human curator. We would like to emphasize on the importance of sharing chronogram data, including systematically curated metadata, into open repositories, such as OpenTree's Phylesystem (McTavish et al., 2015) for the benefit of research and the scientific community as a whole. Another important source of expert data on time of lineage divergence is TimeTree's database (Hedges, Dudley, & Kumar, 2006), which holds chronograms from more than 4k published studies, and is fully browsable using its graphical user interface (timetree.org). TimeTree's chronogram database was not accessible in computer readable format until very recently (Kumar et al., 2022), when its terms of use and website application were updated, now allowing some kinds of reuse, but not redistribution.

⁴¹⁶   The inaccessibility of TimeTree's database was an inspiration for the DateLife

⁴¹⁷ project, which was born as a prototype tool initially developed over a series of hackathons

⁴¹⁸ at the National Evolutionary Synthesis Center, NC, USA (Stoltzfus et al., 2013), as the

⁴¹⁹ need to make scientific information that is funded by the public practically available to the

⁴²⁰ public was acknowledged and prioritized.

⁴²¹   As we envision that DateLife will have many interesting applications in research and

⁴²² beyond, we emphasize that DateLife's results –as well as any insights gleaned from them,

⁴²³ largely depend on the quality of the source chronograms: low quality chronograms will

⁴²⁴ produce low quality results. The "garbage in, garbage out" problem has long been recognised

⁴²⁵ in supertree methods for summarizing phylogenetic trees (Bininda-Emonds et al., 2004). We

⁴²⁶ note that this is a surfacing issue of any automated tool for biological data analysis. For

⁴²⁷ example, DNA riddled with sequencing errors will produce generally poor alignments that

⁴²⁸ will return biased evolutionary hypothesis, independently of the quality of the analysis

⁴²⁹ software used. Again, we urge readers and DateLife users to explore all input chronograms

⁴³⁰ before using a summary chronogram resulting from a DateLife workflow.

⁴³¹   Finally, uncertainty and variability of chronogram node age estimates might pose larger

⁴³² issues in some research areas than others. For example, in ecological and conservation

⁴³³ biology studies, it has been shown that incorporating some chronogram data provides better

⁴³⁴ results than when not using any age data at all, even if the node ages are not good quality

⁴³⁵ (Webb et al., 2008). In the following sections we discuss the particularities of divergence

⁴³⁶ times from DateLife's summary chronograms and their impact on certain evolutionary

⁴³⁷ analyses, for consideration of the readers and users in different research areas.

⁴³⁸   *Age Variation in Source Chronograms*

⁴³⁹   Conflict in estimated ages among alternative studies is common in the literature. See,

⁴⁴⁰ for example, the robust ongoing debate about crown group age of angiosperms

(Barba-Montoya, Reis, Schneider, Donoghue, & Yang, 2018; Magallón, Gómez-Acevedo,

Sánchez-Reyes, & Hernández-Hernández, 2015; Ramshaw et al., 1972; Sanderson & Doyle,

2001; Sauquet, Ramírez-Barahona, & Magallón, 2021). Alternative source chronograms

available for the same taxa have potentially been estimated implementing different types of

calibrations, which affects the resulting node age estimates. For example, in the DateLife

analysis of the Fringillidae shown above, the chronograms from one study (Burns et al.,

2014) were inferred using molecular substitution rate estimates across birds (Weir &

Schluter, 2008), and have much older age estimates for the same nodes than chronograms

that were inferred using fossil calibrations (Figs. ~~5, 6;~~ 3, 4c, Supplementary Figs. ~~S1c, S4~~S4c,

S10). Another source of conflict in estimated node ages can arise from different placements

for the same calibration, which would imply fundamentally distinct evolutionary hypotheses

(Antonelli et al., 2017). For example, two independent researchers working on the same clade

should both carefully select and justify their choices of fossil calibration placement. Yet, if

one researcher concludes that a fossil should calibrate the ingroup of a clade, while another

researcher concludes that the same fossil should calibrate the outgroup of the clade, the

resulting age estimates will differ, as the placement of calibrations as stem or crown group is

known to significantly affect estimates of time of lineage divergence (Sauquet, 2013). Finally,

placement of calibrations also affects uncertainty of node age estimates. For example, nodes

that are sandwiched between a calibrated node and a calibrated root have less freedom of

movement and hence narrower confidence intervals (Vos & Mooers, 2004), which inflates

precision for nodes without calibrations but does not necessarily improve accuracy of the

estimated ages.

DateLife's summary chronograms are intended to represent all variation in estimated

node ages from source chronograms. Node age distribution ranges allow to visually explore

ages from source chronograms individually and contextualize and compare them against

other chronograms. Researchers that wish to use summary chronograms in downstream

evolutionary analysis may select multiple trees sampled from the summary distribution of

468   node ages, to account for variation in source chronograms.

469   *Primary vs Secondary Calibrations*

470   DateLife constructs summary chronograms using node ages extracted from existing

471   chronograms, i.e. secondary calibrations. In general, the scientific community has more

472   confidence in chronograms using primary calibrations, where the dated tree is generated from

473   a single analysis where carefully chosen fossil calibrations are the source of absolute time

474   information, than in analyses dated using secondary calibrations (Antonelli et al., 2017;

475   Garzón-Orduña, Silva-Brandão, Willmott, Freitas, & Brower, 2015; Graur & Martin, 2004;

476   Sauquet, 2013; Sauquet et al., 2012; Schenk, 2016; Shaul & Graur, 2002). However,

477   implementation of primary calibrations is difficult: it requires specialized expertise and

478   training to discover, place and apply calibrations appropriately (Hipsley & Müller, 2014;

479   Ksepka et al., 2011). One approach is to use fossils that have been widely discussed and

480   previously curated as calibrations to date other trees (Ksepka et al., 2011; Sauquet, 2013),

481   and making sure that all data reflect a coherent evolutionary history (Sauquet, 2013), as for

482   example done by Antonelli et al. (2017). The Fossil Calibration Database provides data for

483   220 primary calibration points encompassing flowering plants and metazoans, that have been

484   curated by experts and used for dating analysis in peer-reviewed publications (Ksepka et al.,

485   2015). This database facilitates the use of expert primary fossil calibrations in new

486   phylogenetic dating analyses. Yet, users still require the expertise to locate and calibrate

487   appropriate nodes in their phylogenies which correspond with fossils available in the

488   database.

489   Recently, Powell, Waskin, and Battistuzzi (2020) showed in a simulation study that

490   secondary calibrations using node ages based on previous molecular clock analyses can be as

491   good as primary calibrations. Using several secondary calibrations (as opposed to just one)

492   can provide sufficient information to alleviate or even neutralize potential biases (Graur &

493   Martin, 2004; Sauquet, 2013; Shaul & Graur, 2002). Our cross validation analysis also

provides insight into the application of secondary calibrations. Node ages summarized with DateLife and those from the original studies are well correlated (Supplementary Figs. ~~S2-S20~~S5-S23). We also note that DateLife estimates for nodes closer to the root tend to be slightly younger than ages from the original studies. In contrast, nodes closer to the tips tend to be slightly older when estimated using our secondary calibrations than ages from the original studies. The only exception to this trend was observed in Burns et al. (2014) chronogram, which generally displays much younger node ages when estimated using secondary calibrations than the original study (Supplementary Figs. ~~S1, S5~~S4c, S10), supporting previous observations (Sauquet et al., 2012; Schenk, 2016). However, these younger dates are more likely an example of how multiple secondary calibrations can correct erroneous estimates, as dates on the Burns et al. (2014) tree were obtained using a single secondary calibration based on a previously estimated molecular evolution rate across birds from Weir and Schluter (2008), and appear as major outliers compared to alternate estimates for the same nodes based on primary fossil calibrations (Fig. ~~5).~~

~~Further research is needed to fully understand the effects of using secondary calibrations and the use of resulting chronograms in downstream analyses (Hipsley & Müller, 2014; Powell et al. , 2020; Schenk, 2016; Shaul & Graur, 2002~~3, Supplementary Fig. S3).

Sumarizing Chronograms

By default, DateLife currently summarizes all source chronograms that overlap with at least two species names. Users can exclude source chronograms if they have reasons to do so. Strictly speaking, a good chronogram should reflect the real time of lineage divergence accurately and precisely. To our knowledge, there are no tested measures to determine independently when a chronogram is better than another. Yet, several characteristics of the data used for dating analyses, as well as from the output chronogram itself, could be used to score the quality of source chronograms.

520 Some measures that have been proposed are the proportion of lineage sampling and the

521 number of calibrations used (Magallón, 2010; Magallón et al., 2015). Some characteristics

522 that are often cited in published studies as a measure of improved age estimates as compared

523 to previously published estimates are: quality of alignment (missing data, GC content),

524 lineage sampling (strategy and proportion), phylogenetic and dating inference method,

525 number of fossils used as calibrations, support for nodes and ages, and magnitude of

526 confidence intervals.

527 DateLife provides an opportunity to capture concordance and conflict among date

528 estimates, which can also be used as a metric for chronogram reliability. Its open database of

529 chronograms allows other researchers to do such analyses themselves reproducibly, and

530 without needing permission. Though, of course, they should follow proper citation practices,

531 especially for the source chronogram studies.

532 The exercise of summarizing age data from across multiple studies is a common

533 resource in research, as it provides the opportunity to work with a chronogram that reflects

534 a unified evolutionary history for a lineage, by putting together evidence from different

535 hypotheses. ~~The~~ For example, the largest, and taxonomically broadest chronogram currently

536 available from OpenTree was constructed summarizing age data from 2,274 published

537 chronograms using NCBI's taxonomic tree as backbone (Hedges et al., 2015). ~~A~~

538 ~~summarizing exercise may also~~ , which has been widely reused for research. Finally, we

539 note that summarizing chronograms should be done with caution, as it may amplify the

540 effect of uncertainty and errors in source data, and blur parts of the evolutionary history of a

541 lineage that might only be reflected in source chronograms and lost on the summary

542 chronogram (Sauquet et al., 2021).

543 *Effects of Taxon Sampling on Downstream Analyses*

544 Analysis of species diversification of simulated and empirical phylogenies suggest that

545 using a more completely sampled phylogeny provides estimates that are closer to the true

546 diversification history than when analysing incompletely sampled phylogenies (Chang,

547 Rabosky, & Alfaro, 2020; Cusimano, Stadler, & Renner, 2012; Sun et al., 2020). Ideally,

548 phylogenies should be completed using genetic data, but this is a time-consuming and

549 difficult task to achieve for many biological groups. Hence, DateLife's workflow features

550 different ways of assigning divergence times to taxa with missing the absence of branch

551 length data and calibrations and branch lengths for certain taxa.

552   Completing a phylogeny using a stochastic birth-death polytomy resolver and a

553 backbone taxonomy is a common practice in scientific publications: Jetz et al. (2012),

554 created a chronogram of all 9,993 bird species, where 67% had molecular data and the rest

555 was simulated; Rabosky et al. (2018) created a chronogram of 31,536 ray-finned fishes, of

556 which only 37% had molecular data; Smith and Brown (2018) constructed a chronogram of

557 353,185 seed plants where only 23% had molecular data. ~~These stochastically resolved~~

558 ~~chronograms~~ <u>Stochastically resolved chronograms can</u> return diversification rates estimates

559 that appear less biased than those estimated from their incompletely sampled counterparts,

560 even with methods that account for missing lineages by using sampling fractions (Chang et

561 al., 2020; Cusimano et al., 2012), but can also introduce spurious patterns of early bursts of

562 diversification (Cusimano & Renner, 2010; Sun et al., 2020).

563   Taxonomy-based stochastic polytomy resolvers also introduce topological differences in

564 phylogenetic trees. The study of macroevolutionary processes largely depends on an

565 understanding of the timing of species diversification events, and different phylogenetic and

566 chronogram hypothesis can provide very different overviews of the macroevolutionary history

567 of a biological group. For example, alternative topologies in chronograms from the same

568 biological group can infer very different species diversification patterns (Rabosky, 2015; Title

569 & Rabosky, 2016). Similarly, there are worries that patterns of morphological evolution

570 cannot be accurately inferred with phylogenies that have been resolved stochastically over a

taxonomic backbone, as any patterns would be erased by randomization (Rabosky, 2015). We note that the same applies for geography- and morphology-dependent diversification analysis. Hence, we suggest that phylogenies that have been processed with taxonomy-based stochastic polytomy resolvers, including certain summary chronograms from a DateLife analysis, can be useful as null or neutral models, representing the case of a diversification process that is independent of traits and geographical scenario.

Taxonomy-based stochastic polytomy resolvers have been used to advance research in evolution, still, risks come with this practice. Taken to the extreme, one could generate a fully resolved, calibrated tree of all modern and extinct taxa using a single taxonomy, a single calibration, and assigning branch lengths following a birth-death diversification model. Clearly, this can lead to a misrepresentation of the true evolutionary history. We urge DateLife users to follow the example of the large tree papers cited above, by carefully considering the statistical assumptions being made, potential biases, and assessing the consistency of DateLife's results with prior work.

Conclusions

Knowledge of the evolutionary time frame of organisms is key to many research areas: trait evolution, species diversification, biogeography, macroecology and more. It is also crucial for education, science communication and policy, but generating chronograms is difficult, especially for those who want to use phylogenies but who are not systematists, or do not have the time to acquire and develop the necessary knowledge and skills to construct them on their own. Importantly, years of primarily publicly funded research have resulted in vast amounts of chronograms that are already available in scientific publications, but functionally hidden from the public and scientific community for reuse.

The DateLife project allows for easy and fast summarization of public and state-of-the-art data on time of lineage divergence. It is available as an R package, and as a

web-based R shiny application at www.datelife.org. DateLife provides a straightforward way

to get an informed picture of the state of knowledge ~~of~~ for the time frame of evolution of

different regions of the tree of life, and allows identifying regions that require more research,

or that have conflicting information. Additionally, both summary and newly generated trees

using the DateLife workflow are useful to evaluate evolutionary hypotheses in different areas

of research. We hope that the DateLife project will increase awareness of the existing

variation in expert estimations of time of divergence, and foster exploration of the effect of

alternative divergence time hypotheses on the results of analyses, nurturing a culture of more

cautious interpretation of evolutionary results.

Availability

The DateLife software is free and open source. It can be used online through its R

shiny web application hosted at http://www.datelife.org, and locally through the `datelife`

R package, available from Zenodo (https://doi.org/10.5281/zenodo.593938 and the CRAN

repository (Sanchez-Reyes et al., 2022). DateLife's web application is maintained using

RStudio's shiny server and the shiny package open infrastructure, as well as Docker and

OpenTree's infrastructure (datelife.opentreeoflife.org). `datelife`'s stable version can be

installed from the CRAN repository using the command `install.packages(pkgs =`

`"datelife")` from within R. Development versions are available from DateLife's GitHub

repository (https://github.com/phylotastic/datelife) and can be installed using the

command `devtools::install_github("phylotastic/datelife")`.

Supplementary Material

Supplementary Figures can be viewed and downloaded from their Zenodo repository

(https://doi.org/10.5281/zenodo.6683667). Supplementary material, including code,

biological examples, benchmark results, data files and online-only appendices, can be

downloaded from the Dryad data repository (https://doi.org/10.5061/dryad.cnp5hqc6w), as

621 well as in the Zenodo stable repositories that host the reproducible manuscript

622 (https://doi.org/10.5281/zenodo.7435094), the biological examples

623 (https://doi.org/10.5281/zenodo.7435101), and the software benchmark

624 (https://doi.org/10.5281/zenodo.7435106). Development versions corresponding to all of the

625 above are hosted on GitHub, accesible at https://github.com/LunaSare/datelifeMS1,

626 https://github.com/LunaSare/datelife_examples, and

627 https://github.com/LunaSare/datelife_benchmark.

References

Agnarsson, I., & Miller, J. A. (2008). Is ACCTRAN better than DELTRAN? *Cladistics*, *24*(6), 1032–1038.

Alström, P., Hooper, D. M., Liu, Y., Olsson, U., Mohan, D., Gelang, M., . . . Price, T. D. (2014). Discovery of a relict lineage and monotypic family of passerine birds. *Biology Letters*, *10*(3), 20131067.

Ané, C., Eulenstein, O., Piaggio-Talice, R., & Sanderson, M. J. (2009). Groves of phylogenetic trees. *Annals of Combinatorics*, *13*(2), 139–167.

Antonelli, A., Hettling, H., Condamine, F. L., Vos, K., Nilsson, R. H., Sanderson, M. J., . . . Vos, R. A. (2017). Toward a self-updating platform for estimating rates of speciation and migration, ages, and relationships of Taxa. *Systematic Biology*, *66*(2), 153–166. https://doi.org/10.1093/sysbio/syw066

Archie, J., Day, W. H., Felsenstein, J., Maddison, W., Meacham, C., Rohlf, F. J., & Swofford, D. (1986). The Newick tree format. Retrieved from {https://evolution.genetics.washington.edu/phylip/newicktree.html}

Avibase. (2022). Yellow-throated Bunting. *Avibase - the World Bird Database*, (Online Resource). Retrieved from {https://avibase.bsc-eoc.org/species.jsp?lang=EN&avibaseid=82D1EE0049D8D927}

Bapst, D. W. (2012). Paleotree: An R package for paleontological and phylogenetic analyses of evolution. *Methods in Ecology and Evolution*, *3*(5), 803–807. https://doi.org/10.1111/j.2041-210X.2012.00223.x

Barba-Montoya, J., Reis, M. dos, Schneider, H., Donoghue, P. C., & Yang, Z. (2018). Constraining uncertainty in the timescale of angiosperm evolution and the veracity of

a cretaceous terrestrial revolution. *New Phytologist*, *218*(2), 819–834.

Barker, F. K. (2014). Mitogenomic data resolve basal relationships among passeriform and passeridan birds. *Molecular Phylogenetics and Evolution*, *79*, 313–324.

Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M., & Lovette, I. J. (2013). Going to extremes: Contrasting rates of diversification in a recent radiation of new world passerine birds. *Systematic Biology*, *62*(2), 298–320.

Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M., & Lovette, I. J. (2015). New insights into new world biogeography: An integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers, and allies. *The Auk: Ornithological Advances*, *132*(2), 333–348.

Barker, F. K., Cibois, A., Schikler, P., Feinstein, J., & Cracraft, J. (2004). Phylogeny and diversification of the largest avian radiation. *Proceedings of the National Academy of Sciences*, *101*(30), 11040–11045.

Beresford, P., Barker, F., Ryan, P., & Crowe, T. (2005). African endemics span the tree of songbirds (passeri): Molecular systematics of several evolutionary "enigmas". *Proceedings of the Royal Society B: Biological Sciences*, *272*(1565), 849–858.

Bininda-Emonds, O. R., Jones, K. E., Price, S. A., Cardillo, M., Grenyer, R., & Purvis, A. (2004). Garbage in, garbage out: Data issues in supertree construction. *Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life*, 267–280.

Bodenhofer, U., Bonatesta, E., Horejš-Kainrath, C., & Hochreiter, S. (2015). Msa: An r package for multiple sequence alignment. *Bioinformatics*, *31*(24), 3997–3999.

Boyle, B., Hopkins, N., Lu, Z., Raygoza Garay, J. A., Mozzherin, D., Rees, T., . . . Enquist, B. J. (2013). The taxonomic name resolution service: An online tool for automated

standardization of plant names. *BMC Bioinformatics, 14*(1).

https://doi.org/10.1186/1471-2105-14-16

Britton, T., Anderson, C. L., Jacquet, D., Lundqvist, S., & Bremer, K. (2007). Estimating Divergence Times in Large Phylogenetic Trees. *Systematic Biology, 56*(788777878), 741–752. https://doi.org/10.1080/10635150701613783

Bryson Jr, R. W., Chaves, J., Smith, B. T., Miller, M. J., Winker, K., Pérez-Emán, J. L., & Klicka, J. (2014). Diversification across the new world within the 'blue'cardinalids (aves: Cardinalidae). *Journal of Biogeography, 41*(3), 587–599.

Burleigh, J. G., Kimball, R. T., & Braun, E. L. (2015). Building the avian tree of life using a large-scale, sparse supermatrix. *Molecular Phylogenetics and Evolution, 84*, 53–63.

Burns, K. J., Shultz, A. J., Title, P. O., Mason, N. A., Barker, F. K., Klicka, J., . . . Lovette, I. J. (2014). Phylogenetics and diversification of tanagers (passeriformes: Thraupidae), the largest radiation of neotropical songbirds. *Molecular Phylogenetics and Evolution, 75*, 41–77.

Chamberlain, S. (2018). *bold: Interface to Bold Systems API*. Retrieved from https://CRAN.R-project.org/package=bold

Chamberlain, S. A., & Szöcs, E. (2013). taxize : taxonomic search and retrieval in R [version 2; referees: 3 approved]. *F1000Research, 2*(191), 1–29. https://doi.org/10.12688/f1000research.2-191.v2

Chang, J., Rabosky, D. L., & Alfaro, M. E. (2020). Estimating diversification rates on incompletely sampled phylogenies: Theoretical concerns and practical solutions. *Systematic Biology, 69*(3), 602–611.

Chaves, J. A., Hidalgo, J. R., & Klicka, J. (2013). Biogeography and evolutionary history of

the n eotropical genus s altator (a ves: T hraupini). *Journal of Biogeography*, *40*(11), 2180–2190.

Claramunt, S., & Cracraft, J. (2015). A new time tree reveals earth history's imprint on the evolution of modern birds. *Science Advances*, *1*(11), e1501005.

Criscuolo, A., Berry, V., Douzery, E. J., & Gascuel, O. (2006). SDM: A fast distance-based approach for (super)tree building in phylogenomics. *Systematic Biology*, *55*(5), 740–755. https://doi.org/10.1080/10635150600969872

Cusimano, N., & Renner, S. S. (2010). Slowdowns in diversification rates from real phylogenies may not be real. *Systematic Biology*, *59*(4), 458–464.

Cusimano, N., Stadler, T., & Renner, S. S. (2012). A new method for handling missing species in diversification analysis applicable to randomly or nonrandomly sampled phylogenies. *Systematic Biology*, *61*(5), 785–792.

Delsuc, F., Philippe, H., Tsagkogeorga, G., Simion, P., Tilak, M.-K., Turon, X., . . . Douzery, E. J. (2018). A phylogenomic framework and timescale for comparative studies of tunicates. *BMC Biology*, *16*(1), 1–14.

Eastman, J. M., Harmon, L. J., & Tank, D. C. (2013). Congruification: Support for time scaling large phylogenetic trees. *Methods in Ecology and Evolution*, *4*(7), 688–691. https://doi.org/10.1111/2041-210X.12051

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797.

Felsenstein, J. (1985). Phylogenies and the Comparative Method. *The American Naturalist*, *125*(1), 1–15. Retrieved from http://www.jstor.org/stable/2461605

Forest, F., Savolainen, V., Chase, M. W., Lupia, R., Bruneau, A., & Crane, P. R. (2005).

Teasing apart molecular-versus fossil-based error estimates when dating phylogenetic trees: A case study in the birch family (betulaceae). *Systematic Botany*, *30*(1), 118–133.

Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *The American Naturalist*.

Garzón-Orduña, I. J., Silva-Brandão, K. L., Willmott, K. R., Freitas, A. V., & Brower, A. V. (2015). Incompatible ages for clearwing butterflies based on alternative secondary calibrations. *Systematic Biology*, *64*(5), 752–767.

GBIF Secretariat. (2022). GBIF Backbone Taxonomy. *Checklist dataset*, (Online Resource accessed via GBIF.org). Retrieved from {https://doi.org/10.15468/39omei }

Gibb, G. C., England, R., Hartig, G., McLenachan, P. A., Taylor Smith, B. L., McComish, B. J., . . . Penny, D. (2015). New zealand passerines help clarify the diversification of major songbird lineages during the oligocene. *Genome Biology and Evolution*, *7*(11), 2983–2995.

Graur, D., & Martin, W. (2004). Reading the entrails of chickens: Molecular timescales of evolution and the illusion of precision. *TRENDS in Genetics*, *20*(2), 80–86.

Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R. C., Braun, E. L., Braun, M. J., . . . others. (2008). A phylogenomic study of birds reveals their evolutionary history. *Science*, *320*(5884), 1763–1768.

Harvey, P. H., Pagel, M. D., & others. (1991). *The comparative method in evolutionary biology* (Vol. 239). Oxford university press Oxford.

Hedges, S. B., Dudley, J., & Kumar, S. (2006). TimeTree: A public knowledge-base of divergence times among organisms. *Bioinformatics*, *22*(23), 2971–2972.

763   https://doi.org/10.1093/bioinformatics/btl505

764 Hedges, S. B., Marin, J., Suleski, M., Paymer, M., & Kumar, S. (2015). Tree of life reveals

765   clock-like speciation and diversification. *Molecular Biology and Evolution*, *32*(4),

766   835–845. https://doi.org/10.1093/molbev/msv037

767 Heibl, C. (2008). *PHYLOCH: R language tree plotting tools and interfaces to diverse*

768   *phylogenetic software packages.* Retrieved from

769   http://www.christophheibl.de/Rpackages.html

770 Hipsley, C. A., & Müller, J. (2014). Beyond fossil calibrations: Realities of molecular clock

771   practices in evolutionary biology. *Frontiers in Genetics*, *5*, 138.

772 Hooper, D. M., & Price, T. D. (2017). Chromosomal inversion differences correlate with

773   range overlap in passerine birds. *Nature Ecology & Evolution*, *1*(10), 1526.

774 Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic

775   trees. *Bioinformatics*, *17*(8), 754–755.

776   https://doi.org/10.1093/bioinformatics/17.8.754

777 Jetz, W., Thomas, G., Joy, J. J., Hartmann, K., & Mooers, A. (2012). The global diversity

778   of birds in space and time. *Nature*, *491*(7424), 444–448.

779   https://doi.org/10.1038/nature11631

780 Johansson, U. S., Fjeldså, J., & Bowie, R. C. (2008). Phylogenetic relationships within

781   passerida (aves: Passeriformes): A review and a new molecular phylogeny based on

782   three nuclear intron markers. *Molecular Phylogenetics and Evolution*, *48*(3), 858–876.

783 Katoh, K., Asimenos, G., & Toh, H. (2009). Multiple alignment of dna sequences with mafft.

784   In *Bioinformatics for dna sequence analysis* (pp. 39–64). Springer.

785 Kimball, R. T., Oliveros, C. H., Wang, N., White, N. D., Barker, F. K., Field, D. J., . . .

others. (2019). A phylogenomic supertree of birds. *Diversity*, *11*(7), 109.

Klicka, J., Barker, F. K., Burns, K. J., Lanyon, S. M., Lovette, I. J., Chaves, J. A., & Bryson
    Jr, R. W. (2014). A comprehensive multilocus assessment of sparrow (aves:
    Passerellidae) relationships. *Molecular Phylogenetics and Evolution*, *77*, 177–182.

Ksepka, D. T., Benton, M. J., Carrano, M. T., Gandolfo, M. A., Head, J. J., Hermsen, E. J.,
    . . . others. (2011). *Synthesizing and databasing fossil calibrations: Divergence dating
    and beyond*. The Royal Society.

Ksepka, D. T., Parham, J. F., Allman, J. F., Benton, M. J., Carrano, M. T., Cranston, K.
    A., . . . others. (2015). The fossil calibration database—a new resource for divergence
    dating. *Systematic Biology*, *64*(5), 853–859.

Kumar, S., Suleski, M., Craig, J. M., Kasprowicz, A. E., Sanderford, M., Li, M., . . .
    Hedges, S. B. (2022). TimeTree 5: An expanded resource for species divergence
    times. *Molecular Biology and Evolution*, *39*(8), msac174.

Lamichhaney, S., Berglund, J., Almén, M. S., Maqbool, K., Grabherr, M., Martinez-Barrio,
    A., . . . others. (2015). Evolution of darwin's finches and their beaks revealed by
    genome sequencing. *Nature*, *518*(7539), 371–375.

Laubichler, M. D., & Maienschein, J. (2009). *Form and function in developmental evolution*.
    Cambridge University Press.

Lepage, D. (2004). *Avibase: The world bird database*. Bird Studies Canada.

Lepage, D., Vaidya, G., & Guralnick, R. (2014). Avibase–a database system for managing
    and organizing taxonomic concepts. *ZooKeys*, (420), 117.

Lerner, H. R., Meyer, M., James, H. F., Hofreiter, M., & Fleischer, R. C. (2011). Multilocus
    resolution of phylogeny and timescale in the extant adaptive radiation of hawaiian

809   honeycreepers. *Current Biology*, *21*(21), 1838–1844.

810 Lovette, I. J., Pérez-Emán, J. L., Sullivan, J. P., Banks, R. C., Fiorentino, I.,

811   Córdoba-Córdoba, S., . . . others. (2010). A comprehensive multilocus phylogeny for

812   the wood-warblers and a revised classification of the parulidae (aves). *Molecular*

813   *Phylogenetics and Evolution*, *57*(2), 753–770.

814 Magallon, S., & Sanderson, M. (2001). Absolute diversification rates in angiosperm clades.

815   *Evolution*, *55*(9), 1762–1780.

816 Magallón, S. (2010). Using fossils to break long branches in molecular dating: A comparison

817   of relaxed clocks applied to the origin of angiosperms. *Systematic Biology*, *59*(4),

818   384–399.

819 Magallón, S., Gómez-Acevedo, S., Sánchez-Reyes, L. L., & Hernández-Hernández, T. (2015).

820   A metacalibrated time-tree documents the early rise of flowering plant phylogenetic

821   diversity. *New Phytologist*, *207*(2), 437–453.

822 McTavish, E. J., Hinchliff, C. E., Allman, J. F., Brown, J. W., Cranston, K. A., Holder, M.

823   T., . . . Smith, S. (2015). Phylesystem: A git-based data store for community-curated

824   phylogenetic estimates. *Bioinformatics*, *31*(17), 2794–2800.

825 Michonneau, F., Brown, J. W., & Winter, D. J. (2016). rotl: an R package to interact with

826   the Open Tree of Life data. *Methods in Ecology and Evolution*, *7*(12), 1476–1481.

827   https://doi.org/10.1111/2041-210X.12593

828 Morlon, H. (2014). Phylogenetic approaches for studying diversification. *Ecology Letters*,

829   *17*(4), 508–525. https://doi.org/10.1111/ele.12251

830 Moyle, R. G., Oliveros, C. H., Andersen, M. J., Hosner, P. A., Benz, B. W., Manthey, J. D.,

831   . . . Faircloth, B. C. (2016). Tectonic collision and uplift of wallacea triggered the

global songbird radiation. *Nature Communications*, *7*(1), 1–7.

Oliveros, C. H., Field, D. J., Ksepka, D. T., Barker, F. K., Aleixo, A., Andersen, M. J., . . .
others. (2019). Earth history and the passerine superradiation. *Proceedings of the
National Academy of Sciences*, *116*(16), 7916–7925.

Ooms, J., & Chamberlain, S. (2018). *Phylocomr: Interface to 'phylocom'.* Retrieved from
https://CRAN.R-project.org/package=phylocomr

Open Tree Of Life, Redelings, B., Cranston, K. A., Allman, J., Holder, M. T., & McTavish,
E. J. (2016). Open Tree of Life APIs v3.0. *Open Tree of Life Project*, (Online
Resources). Retrieved from
{https://github.com/OpenTreeOfLife/germinator/wiki/Open-Tree-of-Life-Web-
APIs}

Open Tree Of Life, Redelings, B., Sánchez Reyes, L. L., Cranston, K. A., Allman, J., Holder,
M. T., & McTavish, E. J. (2019). Open tree of life synthetic tree v12.3. *Zenodo*.
Retrieved from https://doi.org/10.5281/zenodo.3937742

Ödeen, A., Håstad, O., & Alström, P. (2011). Evolution of ultraviolet vision in the largest
avian radiation-the passerines. *BMC Evolutionary Biology*, *11*(1), 1–8.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: analyses of phylogenetics and
evolution in R language. *Bioinformatics*, *20*(2), 289–290.

Parchman, T. L., Benkman, C. W., & Mezquida, E. T. (2007). Coevolution between
hispaniolan crossbills and pine: Does more time allow for greater phenotypic
escalation at lower latitude? *Evolution*, *61*(9), 2142–2153.

Päckert, M., Martens, J., Sun, Y.-H., Severinghaus, L. L., Nazarenko, A. A., Ting, J., . . .
Tietze, D. T. (2012). Horizontal and elevational phylogeographic patterns of

himalayan and southeast asian forest passerines (aves: Passeriformes). *Journal of Biogeography*, *39*(3), 556–573.

Pennell, M. W., Eastman, J. M., Slater, G. J., Brown, J. W., Uyeda, J. C., FitzJohn, R. G., . . . Harmon, L. J. (2014). Geiger v2. 0: An expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics*, *30*(15), 2216–2218.

Posadas, P., Crisci, J. V., & Katinas, L. (2006). Historical biogeography: A review of its basic concepts and critical issues. *Journal of Arid Environments*, *66*(3), 389–403.

Powell, A. F., Barker, F. K., Lanyon, S. M., Burns, K. J., Klicka, J., & Lovette, I. J. (2014). A comprehensive species-level molecular phylogeny of the new world blackbirds (icteridae). *Molecular Phylogenetics and Evolution*, *71*, 94–112.

Powell, C. L. E., Waskin, S., & Battistuzzi, F. U. (2020). Quantifying the error of secondary vs. Distant primary calibrations in a simulated environment. *Frontiers in Genetics*, *11*, 252.

Price, T. D., Hooper, D. M., Buchanan, C. D., Johansson, U. S., Tietze, D. T., Alström, P., . . . others. (2014). Niche filling slows the diversification of himalayan songbirds. *Nature*, *509*(7499), 222.

Pulgarín-R, P. C., Smith, B. T., Bryson Jr, R. W., Spellman, G. M., & Klicka, J. (2013). Multilocus phylogeny and biogeography of the new world pheucticus grosbeaks (aves: Cardinalidae). *Molecular Phylogenetics and Evolution*, *69*(3), 1222–1227.

Rabosky, D. L. (2015). No substitute for real data: A cautionary note on the use of phylogenies from birth–death polytomy resolvers for downstream comparative analyses. *Evolution*, *69*(12), 3207–3216.

Rabosky, D. L., Chang, J., Title, P. O., Cowman, P. F., Sallan, L., Friedman, M., . . . others.

878   (2018). An inverse latitudinal gradient in speciation rate for marine fishes. *Nature*,

879   *559*(7714), 392.

880   Ramshaw, J., Richardson, D., Meatyard, B., Brown, R., Richardson, M., Thompson, E., &

881   Boulter, D. (1972). The time of origin of the flowering plants determined by using

882   amino acid sequence data of cytochrome c. *New Phytologist*, *71*(5), 773–779.

883   Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The barcode of life data system

884   (http://www. Barcodinglife. Org). *Molecular Ecology Notes*, *7*(3), 355–364.

885   R Core Team. (2018). *R: a language and environment for statistical computing.* Vienna,

886   Austria: R Foundation for Statistical Computing.

887   Rees, & Cranston, K. (2017). Automated assembly of a reference taxonomy for phylogenetic

888   data synthesis. *Biodiversity Data Journal*, (5).

889   Rees, Vandepitte, L., Decock, W., & Vanhoorne, B. (2017). IRMNG 2006–2016: 10 Years of

890   a Global Taxonomic Database. *Biodiversity Informatics*, *12*.

891   Revell, L. J. (2012). Phytools: An r package for phylogenetic comparative biology (and other

892   things). *Methods in Ecology and Evolution*, *3*, 217–223.

893   Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference

894   under mixed models. *Bioinformatics*, *19*(12), 1572–1574.

895   https://doi.org/10.1093/bioinformatics/btg180

896   Roquet, C., Lavergne, S., & Thuiller, W. (2014). One tree to link them all: A phylogenetic

897   dataset for the european tetrapoda. *PLoS Currents*, *6*.

898   Sanchez-Reyes, L. L., & O'Meara, B. (2022). `datelifeplot`: Methods to plot chronograms

899   and outputs of the `datelife` package. *R Package Release V0.2.2.* Retrieved from

900   https://zenodo.org/badge/latestdoi/381501451

901 Sanchez-Reyes, L. L., O'Meara, B., Eastman, J., Heath, T., Wright, A., Schliep, K., . . .

902   Alfaro, M. (2022). `datelife`: Scientific Data on Time of Lineage Divergence for Your

903   Taxa. In *R package version 0.6.6*. Retrieved from

904   https://CRAN.R-project.org/package=datelife and

905   https://doi.org/10.5281/zenodo.593938

906 Sanderson, M. (2002). Estimating Absolute Rates of Molecular Evolution and Divergence

907   Times: A Penalized Likelihood Approach. *Molecular Biology and Evolution*, *19*(1),

908   101–109. https://doi.org/10.1093/oxfordjournals.molbev.a003974

909 Sanderson, M., & Doyle, J. (2001). Sources of error and confidence intervals in estimating

910   the age of angiosperms from rbcL and 18S rDNA data. *American Journal of Botany*,

911   *88*(8), 1499–1516.

912 Sauquet, H. (2013). A practical guide to molecular dating. *Comptes Rendus Palevol*, *12*(6),

913   355–367.

914 Sauquet, H., Ho, S. Y. W., Gandolfo, M. a, Jordan, G. J., Wilf, P., Cantrill, D. J., . . .

915   Udovicic, F. (2012). Testing the impact of calibration on molecular divergence times

916   using a fossil-rich group: the case of Nothofagus (Fagales). *Systematic Biology*, *61*(2),

917   289–313. https://doi.org/10.1093/sysbio/syr116

918 Sauquet, H., Ramírez-Barahona, S., & Magallón, S. (2021). *The age of flowering plants is*

919   *unknown.*

920 Schenk, J. J. (2016). Consequences of secondary calibrations on divergence time estimates.

921   *PLoS ONE*, *11*(1). https://doi.org/10.1371/journal.pone.0148228

922 Schliep, K. P. (2011). Phangorn: Phylogenetic analysis in r. *Bioinformatics*, *27*(4), 592–593.

923 Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., . . .

924   others. (2020). NCBI Taxonomy: a Comprehensive Update on Curation, Resources

925   and Tools. *Database*, *2020*.

926 Selvatti, A. P., Gonzaga, L. P., & Moraes Russo, C. A. de. (2015). A paleogene origin for

927   crown passerines and the diversification of the oscines in the new world. *Molecular*

928   *Phylogenetics and Evolution*, *88*, 1–15.

929 Shaul, S., & Graur, D. (2002). Playing chicken (gallus gallus): Methodological

930   inconsistencies of molecular divergence date estimates due to secondary calibration

931   points. *Gene*, *300*(1-2), 59–61.

932 Smith, S., & Brown, J. (2018). Constructing a broadly inclusive seed plant phylogeny.

933   *American Journal of Botany*, *105*(3), 302–314.

934 Smith, S., & O'Meara, B. (2012). TreePL: Divergence time estimation using penalized

935   likelihood for large phylogenies. *Bioinformatics*, *28*(20), 2689–2690.

936   https://doi.org/10.1093/bioinformatics/bts492

937 Stoltzfus, A., Lapp, H., Matasci, N., Deus, H., Sidlauskas, B., Zmasek, C. M., . . . Jordan, G.

938   (2013). Phylotastic! Making tree-of-life knowledge accessible, reusable and convenient.

939   *BMC Bioinformatics*, *14*. https://doi.org/10.1186/1471-2105-14-158

940 Sun, M., Folk, R. A., Gitzendanner, M. A., Soltis, P. S., Chen, Z., Soltis, D. E., & Guralnick,

941   R. P. (2020). Estimating rates and patterns of diversification with incomplete

942   sampling: A case study in the rosids. *American Journal of Botany*, *107*(6), 895–909.

943 Tietze, D. T., Päckert, M., Martens, J., Lehmann, H., & Sun, Y.-H. (2013). Complete

944   phylogeny and historical biogeography of true rosefinches (aves: Carpodacus).

945   *Zoological Journal of the Linnean Society*, *169*(1), 215–234.

946 Title, P. O., & Rabosky, D. L. (2016). Do Macrophylogenies Yield Stable Macroevolutionary

Inferences? An Example from Squamate Reptiles. *Systematic Biology*, syw102. https://doi.org/10.1093/sysbio/syw102

Treplin, S., Siegert, R., Bleidorn, C., Thompson, H. S., Fotso, R., & Tiedemann, R. (2008). Molecular phylogeny of songbirds (aves: Passeriformes) and the relative utility of common nuclear marker loci. *Cladistics*, *24*(3), 328–349.

Uyeda, J. C., Pennell, M. W., Miller, E. T., Maia, R., & McClain, C. R. (2017). The evolution of energetic scaling across the vertebrate tree of life. *The American Naturalist*, *190*(2), 185–199.

Vos, R. A., Balhoff, J. P., Caravas, J. A., Holder, M. T., Lapp, H., Maddison, W. P., . . . others. (2012). NeXML: Rich, extensible, and verifiable representation of comparative data and metadata. *Systematic Biology*, *61*(4), 675–689. https://doi.org/10.1093/sysbio/sys025

Vos, R. A., & Mooers, A. Ø. (2004). Reconstructing divergence times for supertrees: A molecular approach. *Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life*, 281–299.

Webb, C. (2000). Exploring the Phylogenetic Structure of Ecological Communities : An Example for Rain Forest Trees. *The American Naturalist*, *156*(2), 145–155.

Webb, C., Ackerly, D., & Kembel, S. (2008). Phylocom: Software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, *24*(18), 2098–2100. https://doi.org/10.1093/bioinformatics/btn358

Webb, C., & Donoghue, M. (2005). Phylomatic: Tree assembly for applied phylogenetics. *Molecular Ecology Notes*, *5*(1), 181–183.

Weir, J., & Schluter, D. (2008). Calibrating the avian molecular clock. *Molecular Ecology*,

970   *17*(10), 2321–2328.

971   Zuccon, D., Prŷs-Jones, R., Rasmussen, P. C., & Ericson, P. G. (2012). The phylogenetic

972   relationships and generic limits of finches (fringillidae). *Molecular Phylogenetics and*

973   *Evolution*, *62*(2), 581–596.