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1 Summary

2 Abstract

1. Phylogenies are a key part of research in all areas of biology. Tools that automatize some parts of the process of phylogenetic reconstruction (mainly character matrix construction) have been developed for the advantage of both specialists in the field of phylogenetics and nonspecialists. However, interpretation of results, comparison with previously available phylogenetic hypotheses, and choosing of one phylogeny for downstream analyses and discussion still impose difficulties to one that is not a specialist either on phylogenetic methods or on a particular group of study.

2. Physcraper is an open-source, command-line Python program that automatizes the update of published phylogenies by making use of public DNA sequence data and taxonomic information, providing a framework for comparison of published phylogenies with their updated versions.

3. Physcraper can be used by the nonspecialist, as a tool to generate phylogenetic hypothesis based on already available expert phylogenetic knowledge. Phylogeneticists and group specialists will find it useful as a tool to facilitate comparison of alternative phylogenetic hypotheses (topologies). ***Is physcraper intended for the nonspecialist?? We have two types of nonspecialists: the ones that do not know about phylogenetic methods and the ones that might know about phylogenetic methods but do not know much about a certain biological group.***

4. Physcraper implements node by node/topology comparison of the the original and the updated trees using the conflict API of OToL, and summarizes differences.

5. We hope the physcraper workflow demonstrates the benefits of opening results in phylogenetics and encourages researchers to strive for better data sharing practices.

6. Physcraper can be used with any OS. Detailed instructions for installation and use are available at https://github.com/McTavishLab/physcraper.

Keywords: phylogeny,

3 Introduction

Phylogenies are important.

Generating phylogenies is not easy and it is largely artisanal. Although many efforts to automatize the process have been done, and the community is using those more and more, automatization of phylogenetic reconstruction is still not a widespread practice and among other benefits, it might be key for adoption of better reproducibility practices in the phylogenetics community. ***paragraph better to end discussion???***

The process of phylogenetic reconstruction implies many steps (that I generalize to the following):

1. Obtention of molecular or morphological character data – get DNA from some organisms and sequence it, or get it from an online nucleotide data repository, such as GenBank (Benson *et al.* 2000; Wheeler *et al.* 2000).
2. Assemble a hypothesis of homology – Create a matrix of your character data, by aligning the sequences, in the case of molecular data. Make sure thay are paralogs!
3. Analyse this hypothesis of homology to infer phylogenetic relationships among the organisms you are studying – Use different available programs to infer molecular evolution, trees and times of divergence.
4. Discuss the inferred relationships in the context of previous hypothesis, the biology and biogeography of the organisms, etc. – Answer the question, *is this phylogenetic solution fair/reasonable?*

Each of these steps require different types of specialized training: in the field, in the lab, in front of a computer, discussions with experts in the methods, and/or in the biological group of study. All of these steps also require considerable amounts of time for training and implementation.

In the past decade, various studies have developed solutions to automatize the first and second steps, by creating pipelines that mine already available molecular data from the GenBank repository (Benson *et al.* 2000; Wheeler *et al.* 2000), to obtain homologous characters that can be used for phylogenetic reconstruction. These tools have been presented as aid for the nonspecialist to decrease some of the difficulties in the generation of phylogenetic knowledge. However, they are not that often used as so, suggesting that there are

still difficulties for the nonspecialist. The phylogenetic community has some reserves towards these tools, too. Mainly because they sometimes act as a black box. However, automatizing the assembly of the character data set is a crucial step towards reproducibility for a task that was otherwise primarily artisanal and hence largely non-reproducible.

Even if it is hard to obtain phylogenies, we invest copious amounts of time and energy in generating them. Issues such as food security, global warming, global health are crucial to solve and phylogenies might help. There is a lot of phylogenetic knowledge already available in published peer-reviewed studies. In this sense, the non-specialists (and also the specialist) face a new problem: how do I choose the best phylogeny.

Public phylogenies can be updated with the ever increasing amount of genetic data that is available on GenBank (Benson *et al.* 2000; Wheeler *et al.* 2000).

We present a way to automatize and standardize the comparison of phylogenetic hypotheses and to allow reproducibility of this last step of the research process.

A key aspect of the standard phylogenetic workflow is comparison with already existing phylogenetic hypotheses and with phylogenies that are considered "best" by experts not only in phylogenetics, but also experts on the focal group of study.

Concerns I think people have about these tools: - Errors in identification of sequences - Little control along the process - Too much of a black box?

Most of these phylogenies are being constructed by people learning about the methods, so they want to know what is going on.

The pipelines are so powerful and they will give you an answer, but there is no way to assess if it is better than previous answers, it just assumes it is better because it used more data.

All these pipelines start tree construction from zero? Yes.

The goal of Physcraper is to build upon previous phylogenetic knowledge, allowing a direct comparison

between existing phylogenies and phylogenies that are constructed using new genetic data retrieved from a public nucleotide database (i.e., GenBank (Benson *et al.* 2000; Wheeler *et al.* 2000)).

To achieve this, Physcraper uses the Open Tree of Life phylesystem and connects it to the TreeBase database, to (1) get the original DNA data set matrices (alignments) that produced a phylogeny that was published and then made available in the OToL database, (2) use this DNA alignments as a starting point to get new genetic data belonging to the focal group of study, to (3) finally update the phylogenetic relationships in the group.

A less automated workflow is one in which the alignments that generated the published phylogeny are stored in other public database (such as DRYAD) or elsewhere (the users computer), and are provided by the users.

The original tree is by default used as starting tree for the phylogenetic searches, but it can also be set as a full topological constraint or not used at all, depending on the goals of the user.

Physcraper implements node by node comparison of the the original and the updated trees, using the conflict API of OToL.

4 How does Physcraper work?

4.1 The input: a study tree and an alignment

- The study tree is a published phylogenetic tree stored in the OToL database, phylesystem (McTavish *et al.* 2015). The main reason for this is that trees in phylesystem have a set of user defined characteristics that are essential for automatizing the phylogeny update process. The most relevant of these being the definition of ingroup and outgroup. Outgroup and ingroup taxa in the original tree are identified and tagged. This allows to automatically set the root for the updated tree on the next steps of the pipeline. A user can choose from the 'r rotl::tol_about()$num_source_trees' published trees supporting the resolved node of the synthetic tree in the OToL website (<>). If the tree you are interested in updating is not in there, you can upload it via OToL's curator tool (<https://tree.opentreeoflife.org/curator).

- The alignment should be a gene alignment that was used to generate the tree. The original alignments are usually stored in a public repository such as TreeBase (Piel *et al.* 2009; Vos *et al.* 2012), DRYAD (http://datadryad.org/), or the journal were the tree was originally published. If the alignment is stored in TreeBase, `physcraper` can download it directly, either from the TreeBASE website (https://treebase.org/) or through the TreeBASE GitHub repository (SuperTreeBASE; https://github.com/TreeBASE/supertreebase). If the alignment is on another repository, or provided personally by the owner, a copy of it has to be downloaded by the user, and it's local path has to be provided as an argument.
- A taxon name matching step is performed to verify that all taxon names on the tips of the tree are in the DNA character matrix and vice versa.
- A ".csv" file with the summary of taxon name matching is produced for the user.
- Unmatched taxon names are dropped from both the tree and alignment. Technically, just one matching name is needed to perform the searches. Please, see next section.
- A ".tre" file and a ".fas" file containing only the matched taxa are generated and saved in the `inputs` folder to be used in the following steps.

4.2 DNA sequence search and cleaning

- The next step is to identify the search taxon within the reference taxonomy. The search taxon will be used to constraint the DNA sequence search on the nucleotide database within that taxonomic group. Because we are using the NCBI nucleotide database, by default the reference taxonomy is the NCBI taxonomy. The search taxon can be provided by the user. If none is provided, then the search taxon is identified as the Most Recent Common Ancestor (MRCA) of the matched taxa belonging to the ingroup in the tree, that is also a named clade in the reference taxonomy. This is known as the Most Recent Common Ancestral Taxon (MRCAT; also referred in the literature as the Least Inclusive Common Ancestral Taxon - LICA). The MRCAT can be different from the phylogenetic MRCA when the latter is an unnamed clade in the reference taxonomy. To automatically identify the MRCAT of a group of taxon names, we make use of the OToL taxonomy tool (https:

//github.com/OpenTreeOfLife/germinator/wiki/Taxonomy-API-v3#mrca).

Users can provide a search taxon that is either a more or a less inclusive clade relative to the ingroup of the original phylogeny. If the search taxon is more inclusive, the sequence search will be performed outside the MRCAT of the matched taxa, e.g., including all taxa within the family or the order that the ingroup belongs to. If the search taxon is a less inclusive clade, the users can focus on enriching a particular clade/region within the ingroup of the phylogeny.

- The Basic Local Alignment Search Tool, BLAST [Altschul *et al.* (1990); altschul1997gapped] is used to identify similarity between DNA sequences within the search taxon in a nucleotide database, and the accepted sequences on the alignment. The blastn function from the BLAST command line tools (Camacho *et al.* 2009) is used for local-database searches. A modified biopython blast function is used for web-based searches.

- The DNA sequence similarity search can be done on a local database that is easily setup by the user. In this case, the blastn function is used to performs the similarity search (Camacho *et al.* 2009).

- The search can also be performed remotely, on the NCBI database. In this case, the bioPython BLAST function was modified to accepts is used to perform the similarity search.

- A pairwise alignment-against-all BLAST search is performed. This means that each sequence in the alignment is BLASTed against DNA sequences in a nucleotide database constrained to the search taxon. Results from each one of these BLAST runs are recorded, and matched sequences are saved along with their corresponding identification numbers (accesion numbers in the case of the GenBank database). This information will be used later to store the whole sequences in a dedicated library within the physcraper folder, allowing for secondary analyses to run significantly faster.

- Matched sequences below an e-value, percentage similarity, and outside a minimum and maximum length threshold are discarded. ***REPORT THE DEFAULT VALUES AND DESCRIBE WHAT THEY MEAN*** This filtering leaves out genomic sequences. All acepted sequences are asigned an

internal identifier, and are further filtered.

- Because the original alignments usually lack database id numbers, a filtering step is needed. Accepted sequences that belong to the same taxon of the query sequence, and that are either identical or shorter than the original sequence are discarded. Only longer sequences belonging to the same taxon as the orignal sequence will be considered further for analysis.

- Among the remaining filtered sequences, there are usually several exemplars per taxon. Although it can be useful to keep some of them to, for example, investigate monophyly within species, there can be hundreds of exemplar sequences per taxon for some markers. To control the number of sequences per taxon in downstream analyses, 5 sequences per taxon are chosen at random. This number is set by default but can be modified by the user.

- Reverse complement sequences are identified and translated.

- Users can choose to perform a more "cycles" of sequence similarity search, by blasting the newly found sequences. This can be done iteratively, but by default only sequences in the alignment are blasted. ***Is there an argument to control the number of cycles of blast searches with new sequences?***

- Accepted sequences are downloaded in full, and stored as a local database in a directory that is globally accesible (physcraper/taxonomy), so they are accesible for further runs.

- A fasta file containing all filtered and processed sequences resulting from the BLAST search is generated for the user.

4.3 DNA sequence alignment

- The software MUSCLE (Edgar 2004) is implemented to perform alignments.
- First, all new sequences are aligned using default MUSCLE options.
- Then, a MUSCLE profile alignment is performed, in which the original alignment is used as a template to align new sequences. This ensures that the final alignment follows the homology criteria established

by the original alignment.

- The final alignment is not further processed automatically. We encourage users to check it either by eye and perform manual refinement or using any of the many tools for alignment processing, to eliminate columns with no information.

4.4 Tree reconstruction and comparison

- A gene tree is reconstructed for each alignment provided, using a Maximum Likelihood approach implemented with the software RAxML (Stamatakis 2014) with 100 classic rapid bootstrap (Felsenstein 1985) replicates by default. The number of bootsrap replicates can be modified by the user. Other type of bootstrap that I think is not yet incorporated into physcraper is the Transfer Bootstrap Expectation (TBE) recently proposed in Lemoine *et al.* (2018).
- Bootstrap results are summarized with Dendropy ADD CITATION
- The final result is an updated phylogenetic hypothesis for each of the genes provided in the alignment.
- Tips on all trees generated by physcraper are defined by a taxon name space, allowing to perform comparisons and conflict analyses.
- Robinson Foulds weighted and unweighted metrics ARE CALCULATED WITH DENDROPY TOO.
- Describe what a conflict analysis is: Node by node comparison of the resulting clades compared to CITE REDELINGS AND HOLDER (**???** and holder)
- For the conflict analysis to be meaningful, the root of the tree ineeds to be accurately defined.
- A SUGGESTED DEFAULT ROOTING BASED ON THE OPEN TREE TAXONOMY is implemented for now. DESCRIBE HOW IT WORKS. SAY THAT IT IS A PROBLEM. Automatic rooting is not that smart yet. The best way right now is for users to define outgroups so trees are better rooted.
- Currently, the root is determined by finding the parent node of the sequences that do not belong to the ingroup/ search taxon. This ensures a correct rooting of the tree even when the search taxon is more inclusive than the ingroup.
- Conflict information can only be generated in the context of the whole Open Tree of Life. Otherwise, it is not really possible to get conflict data. ***- One way to compare two independent phylogenetic***

trees is to compare them both to the synthetic OToL and then measure how well they do against each other

5 Examples

5.1 The hollies

The genus *Ilex* is the only extant clade within the family Aquifoliaceae, order Aquifoliales of flowering plants. It encompasses between 400-600 living species. A review of litterature shows that there are three published phylogenetic trees, showing relationships within the hollies. The first one has been made available both on OToL phylesystem and synth tree, and on treeBASE, it samples 48 species. The second has not been made available anywhere, not even in supplementary data of the journal. ***Contact authors? They seem old school, probably do not wanna share their data.*** The most recent one has been made available in the OToL Phylesystem and DRYAD. It is the best sampled yet, with 200 species. However, it has not been added to the syntehtic tree yet. This makes it a perfect case to test the basic functionalities of physcraper: we know that the sequences of the most recently published tree have been made available on the GenBank database (Benson *et al.* 2000; Wheeler *et al.* 2000). Updating the oldest tree, we should get something very similar to the newest tree.

5.2 The Ascomycota

Let's be more specific now about our X group and say it is the Ascomycota. The best tree currently available in OToL was published by Schoch *et al.* (2009). The first step, is to get the Open Tree of Life study id. There are some options to do this: - You can go to the Open Tree of Life website and browse until you find it, or - you can get the study id using R tools: - By using the TreeBase ID of the study (which is not fully exposed on the TreeBase website home page of the study, so you have to really look it up manually):

```
rotl::studies_find_studies(property = "treebaseId", value = "S2137")
```

```
##   study_ids n_trees tree_ids candidate study_year title
```

```
## 1    pg_238       1  tree109                  2009
##                                  study_doi
## 1 http://dx.doi.org/10.1093/sysbio/syp020
```

- By using the name of the focal clade of study (but this behaved very differently):

```
rotl::studies_find_studies(property="ot:focalCladeOTTTaxonName", value="Ascomycota")
```

Once we have the study id, we can gather the trees published on that study:

```
rotl::get_tree_ids(rotl::get_study_meta("pg_238"))
```

```
## [1] "tree109"
```

```
rotl::candidate_for_synth(rotl::get_study_meta("pg_238"))
```

```
## NULL
```

```
my_trees <- rotl::get_study("pg_238")
```

Both trees from this study have NA tips.

Let's check what one of the trees looks like:

1. Download the alignment from TreeBase If you are on the TreeBase home page of the study, you can navigate to the matrix tab, and manually download the alignments that were used to reconstruct the trees reported on the study that were also uploaded to TreeBase and to the Open Tree of Life repository. To make this task easier, you can use a command to download everything into your working folder:

```
physcraper_run.py -s pg_238 -t tree109 -o ../physcraper_example/pg_238
```

In this example, all alignments posted on TreeBase were used to reconstruct both trees.

1. With the study id and the alignment files saved locally, we can do a physcraper run with the command:

```
physcraper_run.py -s pg_238 -t tree109 -a treebase_alns/pg_238tree109.aln -as "nexus" -o pg_238
```

5.3 Testudines example

Phylogeny of the Testudines 6 tips from Crawford *et al.* (2012) There is just one tree in OToL. There is just one alignment on treebase with all the 1 145 loci.

```
physcraper_run.py -s pg_2573 -t tree5959 -tb -db ~/branchinecta/local_blast_db/ -o pg_2573
```

6 Discussion

Data repositories hold more information than meets the eye. Besides the actual data, they have other types of information that can be used for the advantage of science.

Usually, initial ideas about the data are changed by analyses. We expect that this new ideas on the data can be registered on data bases, exposing new comers to expert understanding about the data.

There are many tools that are making use of DNA data repositories in different ways. Most of them focus on efficient ways to mine the data – getting the most homologs. Some focus on accurate ways of mining the data - getting real and clean homologs. Others focus on refinement of the alignment. Most focus on generating full trees *de novo*, mainly for regions of the Tree of Life that have no phylogenetic assessment yet in published studies, but also for regions that have been already studied and that have phylogenetic data already.

All these tools are great efforts for advancing towards reproducibility in phylogenetics, a field that has been largely recognised as somewhat artisanal. We propose adding focus to other sources of information available from data repositories. Taking advantage of public DNA data bases have been the main focus. However, phylogenetic knowledge is also accumulating fast in public and open repositories. In this way, the physcraper pipeline can be complemented with other tools that have been developed for other purposes.

We emphasize that physcraper takes advantage of the knowledge and intuition of the expert community to build upon phylogenetic knowledge, using not only data accumulated in DNA repositories, but phylogenetic

knowledge accumulated in tree repositories. This might help generate new phylogenetic data. But physcraper does not seek to generate full phylogenies *de novo*.

Describe again statistics to compare phylogenies provided by physcraper via OpenTreeOfLife. Mention statistics provided by other tools: PhyloExplorer (Ranwez *et al.* 2009). Compare and discuss.

How is physcraper already useful: - to mine targeted sequences, in this way it is similar to baited analyses from PHLAWD and pyPHLAWD. Phylota does not do baited analyses, I think, only clustered analyses. - Finding

How can it be used for the advantage of the field: - rapid phylogenetic placing of newly discovered species, as mentioned in Webb *et al.* (2010) - obtain trees for ecophylogenetic studies, as mentioned in Helmus & Ives (2012) - one day could be used to sistematize nucleotide databases, such as Genbank (Benson *et al.* 2000; Wheeler *et al.* 2000), as mentioned in San Mauro & Agorreta (2010), i.e., curate ncbi taxonomic assignations. - allows to generate custom species trees for downstream analyses, as mentioned in Stoltzfus *et al.* (2013)

Things that physcraper does not do: - analyse the whole GenBank database (Benson *et al.* 2000; Wheeler *et al.* 2000) to find homolog regions suitable to reconstruct phylogenies, as mentioned in Antonelli *et al.* (2017). There are already some very good tools that do that. - provide basic statistics on data availability to assemble molecular datasets, as mentioned by Ranwez *et al.* (2009). Phyloexplorer does this? - it is not a tree repo, as phylota is, mentioned in Deepak *et al.* (2014)

6.1 Tools that automatize any part of the process of phylogenetic reconstruction:

6.1.1 1. Mining DNA databases to generate datasets suitable for phylogenetic reconstruction

Tool	Citation	Cited by	Description	Supermatrix/gene tree/species tree
Phylota	Sanderson *et al.* (2008)	122 studies	finds sets of DNA homologs on the GenBank database; phylogenetic reconstruction	Supermatrix
AMPHORA	Wu & Eisen (2008)	458 studies	baited search; protein markers on phylogenomic data; personal database of genomes or metagenomic data, manually downloaded either from a public database or from private data; phylogenetic reconstruction	Supermatrix
PHLAWD	Smith *et al.* (2009)	234 studies	Baited search of DNA markers on the GenBank database; phylogenetic reconstruction	Supermatrix

Tool	Citation	Cited by	Description	Supermatrix/gene tree/species tree
Unnamed ruby pipeline, only available from supplementary data of the journal	Peters *et al.* (2011)	64 studies	mining public DNA databases, focuses on filtering massive amounts of mined sequences by using established "criteria of compositional homogeneity and defined levels of density and overlap"	Supermatrix
Unnamed	Grant & Katz (2014)	38 studies	predecessor of phylotol; homolog clustering; public and/or personal DNA database; phylogenetic reconstruction; broad taxon analyses; remove contaminant sequences, based on similarity and on phylogenetic position	supermatrix

Tool	Citation	Cited by	Description	Supermatrix/gene tree/species tree
Unnamed	Chesters & Zhu (2014)	10 studies	algorithm that mines GenBank data to delineate species in the insecta. The authors present a nice comparison with the phylota algorithm	Species trees??
PUmPER	Izquierdo-Carrasco *et al.* (2014)	14 studies	perpetual updating with newly added sequences to GenBank	not sure yet
DarwinTree	Meng *et al.* (2015a)	6 studies	predecessor is Phylogenetic Analysis of Land Plants Platform (PALPP), takes data from GenBank, EMBL and DDBJ for land plants only	not sure
NCBIminer	Xu *et al.* (2015)	4 studies	part of darwintree	not sure

Tool	Citation	Cited by	Description	Supermatrix/gene tree/species tree
SUMAC	Freyman (2015)	19 studies	both "baited" analyses and single-linkage clustering methods, as well as a novel means of determining when there are enough overlapping data in the DNA matrix	not sure
STBase	McMahon *et al.* (2015)	7 studies	pipeline for species tree construction and the public database of one million precomputed species trees	species trees
Unnamed	Papadopoulou *et al.* (2015)	17 studies	Automated DNA-based plant identification for large-scale biodiversity assessment	not sure

Tool	Citation	Cited by	Description	Supermatrix/gene tree/species tree
BIR	Kumar *et al.* (2015)	6 studies	blast, align, identify homologs via constructed trees, curate and realign	supermatrix
SUPERSMART	Antonelli *et al.* (2017)	35 studies	baited analyses up to bayesian divergence time estimation	supermatrix
SOPHI	[Chesters (2017)	17 studies	Searches DNA sequence data from repos other than GenBank, such as transcriptomic and barcoding repos	not sure
phyloSkeleton	Guy (2017)	5 studies	focuses on taxon sampling; baited genomic sequences; public database (NCBI and JGI); marker identification	supermatrix

Tool	Citation	Cited by	Description	Supermatrix/gene tree/species tree
OneTwoTree	Drori *et al.* (2018)	7 studies	Web-based, user-friendly, online tool for species-tree reconstruction, based on the *supermatrix paradigm* and retrieves all available sequence data from NCBI GenBank	supermatrix
pyPhlawd	Smith & Walker (2019)	6 studies	baited and clustering analyses	Supermatrix or gene tree

Tool	Citation	Cited by	Description	Supermatrix/gene tree/species tree
Phylotol	Cerón-Romero *et al.* (2019)	5 studies	"phylogenomic pipeline to allow easy incorporation of data from high-throughput sequencing studies, to automate production of both multiple sequence alignments and gene trees, and to identify and remove contaminants. PhyloToL is designed for phylogenomic analyses of diverse lineages across the tree of life", i.e., bacteria and unicellular eukaryotes	supermatrix and gene trees
phylotaR	Bennett *et al.* (2018)	studies		

According to Cerón-Romero *et al.* (2019), PhyLoTA and BIR "focus on the identification and collection of homologous and paralog genes from public databases such as GenBank", while both AMPHORA and PHLAWD "focus on the construction and refinement of robust alignments rather than the collection of homologs."

6.1.2 2. Searching phylogenetic tree databases

PhyloFinder (Chen *et al.* 2008) - cited by 18: a search engine for phylogenetic databases, using trees from TreeBASE - more related to phylotastic's goal than to updating/creating phylogenies

6.1.3 3. Mining phylogenetic tree databases

PhyloExplorer (Ranwez *et al.* 2009) - cited by 21: a python and MySQL based website to facilitate assessment and management of phylogenetic tree collections. It provides "statistics describing the collection, correcting invalid taxon names, extracting taxonomically relevant parts of the collection using a dedicated query language, and identifying related trees in the TreeBASE database".

6.1.4 4. Pipeline for phylogenetic reconstruction

PhySpeTre (Fang *et al.* 2019) - no citations yet - no sequence retrieval, just phylogenetic reconstruction pipeline.

6.1.5 5. getting metadata and not sequences from GenBank.

Datataxa Ruiz-Sanchez *et al.* (2019) - no citations yet - focus on extracting metadata from GenBank sequence information.

6.2 Phylota overview

Phylota was published as a website to summarize and browse the phylogenetic potential of the GenBank database (Sanderson *et al.* 2008).

Since then, it has been cited 122 times for different reasons.

1. As an example of a tool that mines GenBank data for phylogenetic reconstruction, or that is useful in any way for phylogenetics:
    - original publication of PHLAWD (Smith *et al.* 2009)
    - an analysis identifying research priorities and data requirements for resolving the red algal tree of

life (Verbruggen *et al.* 2010)

- Beaulieu *et al.* (2012a) cites phylota as an example study of very large and comprehensive phylogeny from mined DNA sequence data, (even if no phylogeny was really published there, only the method to do so)
- a review for ecologists about phylogenetic tools (Roquet *et al.* 2013)
- a study constructing a dated seed plant phylogeny using pyPHLAWD (Smith & Brown 2018)
- a study presenting an "assembly and alignment free" method for phylogenetic reconstruction using genomic data. It aims to be incorporated into a pipeline such as phylota some day (Fan *et al.* 2015).
- nexml format presentation (Vos *et al.* 2012) - cites phylota as a tool that uses stored phyloinformatic data that could benefit from adopting nexml, to increase interoperability.
- a study of fruit evolution, analysing a previously published phylogeny of 8911 tips of the Campanulidae, constructed with PHLAWD (Beaulieu & Donoghue 2013)
- a study of Southeast Asia plant biodiversity inventory (Webb *et al.* 2010) - cites phylota as a tool that would allow rapid phylogenetic placing of newly discovered species, and generation of phylogenetically informed guides for field identification.
- a study of wood density for carbon stock assessments (Flores & Coomes 2011), cites phylota as an initiative to "get supertrees resolved up to species level".
- a study proposing something similar to Open tree but applied only to land plants (Beaulieu *et al.* 2012b)
- an analysis of the phylogenetic diversity-area curve (Helmus & Ives 2012), cited phylota as a method alternative to phylomatic to "obtain plant phylogenetic trees for ecophylogenetic studies".
- a study generating a phylogeny of 6,098 species of vascular plants from China (Chen *et al.* 2016) - uses DarwinTree (Meng *et al.* 2015a) and generates sequence data *de novo* for 781 genera.
- a review of the state of methods and knowledge generated by molecular systematics (San Mauro & Agorreta 2010) cites phylota as a tool "intended to systematize GenBank information for large-scale molecular phylogenetics analysis".

- the first phylotastic paper (Stoltzfus *et al.* 2013) cites phylota as a "phylogeny related resource that provides ways to generate custom species trees for downstream use".
- Antonelli *et al.* (2017) cites phylota as a "pipeline that pre-processes entire GenBank releases in pursuit of sufficiently overlapping reciprocal BLAST hits, which are then clustered into candidate data sets". They also use the PHYLOTA database in its own pipeline.
- Deepak *et al.* (2014) present an algorithm for mining of frequent subtrees (common patterns) in collections of phylogenetic trees, as a way to extract meaningful phylogenetic information from collections of trees when compared to maximum agreement subtrees and majority-rule trees. They cite phylota as one of such tree collections available along with TreeBASE (Piel *et al.* 2009).
- Ranwez *et al.* (2009) cites phylota as a "program providing basic statistics on data availability for molecular datasets". They propose a tool to upload and explore user phylogenies to obtain detailed summary statistics on user tree collections.
- Freyman (2015) cites phylota as a tool that "provides a web interface to view all GenBank sequences within taxonomic groups clustered into homologs" but that does not mine for targeted sequences, as opposed to NCBIminer or PHLAWD. They compare the performance of SUMAC to Phylota. This is also presented in their PhD dissertation (Freyman 2017).
- Chesters & Vogler (2013) cites phylota as a data mining tool that compiles metadata from mining of public DNA databases "for construction of large phylogenetic trees and multiple gene sets" and that the authors have recognised that gene annotations in public databases are insufficient and that careful partitioning of orthologous sequences is needed for supermatrix construction. Chesters & Vogler (2013) present a procedure that minimizes the problem of forming multilocus species units in a large phylogenetic data set using algorithms from graph theory.
- Chesters & Zhu (2014) present an algorithm to delineate species form GenBank DNA data, and cites phylota as a tool that partitions "the contents of a database according to homology", by "grouping of database sequences according to internal criteria", searching "from a standardized set of references [...] patterns in sequence similarity and overlap."
- the paper presenting phylotaR, a pipeline that recreates the phylota output but uses the most

updated GenBank release, and is available in R (Bennett *et al.* 2018), cites phylota as its predecessor and inspiration. The authors mention that phylotaR pipeline mimics phylota's pipeline but with improvements.

- The paper presenging PhyloBase (Jamil 2016), cites phylota as one of its resources to get phylogenies, along with TreeBASE and others.
- The paper presenting STBase, a database of one million precomputed species trees (Deepak 2013; McMahon *et al.* 2015), cites phylota as a databse of gene trees or mul-trees, "trees having multiple sequences with the same taxon name".
- Drori *et al.* (2018) present a Web-based, user-friendly, online tool for species-tree reconstruction, based on the *supermatrix paradigm* and retrieves all available sequence data from NCBI GenBank. They cite phylota in the intro as a tool that is "designed to provide users with precomputed sets of clusters that were assembled through a single-linkage clustering approach and additionally provides precomputed gene trees that were reconstructed for each cluster. In particular, the results obtained by PhyLoTa are taxonomically constrained; that is, all sequences of the most recent common ancestor are collected even if one specifies only part of a clade".
- A study developing a tool to link wikipedia data to NCBI taxonomy (Page 2011) cites phylota as a phylogenetic resource that uses the NCBI taxonomy.
- the study that present DarwinTree (Meng *et al.* 2015a), and all derived studies: the study presenting an approach to screen sequence data for The Platform for Phylogenetic Analysis of Land Plants (PALPP), using the MapReduce paradigm to parallelize BLAST (Yong *et al.* 2010), as well as Gao *et al.* (2011), Li *et al.* (2013), Meng *et al.* (2014), Meng *et al.* (2015c), and Meng *et al.* (2015b), all cite phylota using the exact same introduction and sentence: as one among other "studies based on data mining large numbers of taxa or loci".
- A study presenting a tool to asses gene sequence quality for automatic construction of databases (Meng *et al.* 2012a), as well as their parallelized version using MapReduce (Meng *et al.* 2012b), cite phylota (along with Yong *et al.* (2010)) as a tool that relies on sequence similarity (BLAST) and not taxon name annotations in the database, for mining large numbers of taxa or loci, without

making any control on the quality of the sequencing.

- A review on online plant databases aiming to "provide recommendations for current information managers and developers concerning the user interface and experience; and to provide a picture about the possible directions to take for those in charge of the creation of information at all levels". They cite phylota as a tool allowing researchers "to acces equally and globally, without travel, a [phylogenetic?] model of plants at the kingdom level" (Jones *et al.* 2014).
- a paper aiming to establish an online information system for the legumes and to outline "best practices for development of a legume portal to enable data sharing and a better understanding of what data are available, missing, or erroneous, and ultimately facilitate cross-analyses and collaboration within the legume-systematics community and with other stakeholders" (Bruneau *et al.* 2019), cites phylota (along with supersmart and pyphlawd) as a "pipeline for large-scale retrieval of GenBank data of particular taxa or clades". In their Table 1, they also list phylota as a potential data source for developing a legume portal.
- A study on morphological evolution of electric fish skull, that uses phylotaR to retrieve sequences of the family Apteronotidae, order Gymnotiformes (Evans *et al.* 2019), cites phylota as the inspiration and fundament of phylotaR.
- A phylogenetic revision of the Gymnotidae fish (Teleostei: Gymnotiformes), uses phylotaR to retrieve seqeunces, but cites phylota as "a pipeline that implements BLAST searches to both identify and download sequence clusters for listed taxonomic groups to assemble a robust collection of sequences in a reproducible way based on publicly-available gene sequences while avoiding selection bias on the part of the assembler".
- A master thesis on SearchTree, a "software tool that allows users to query efficiently on an arbitrary user taxon list and returns high scoring matches from approximately one billion phylogenetic trees being constructed from molecular sequence data in GenBank" (Deepak 2010), that seems to be the preliminary work for STBase (McMahon *et al.* 2015), cites phylota as "a standard strategy, to assemble sets of homologous sequences (clusters) from a database of all-against-all BLAST searches, [in which] clusters are constructed in the context of the NCBI taxonomy tree for convenience of

409 display, thus child clusters are contained within parent clusters, following the NCBI hierarchy". In
410 opposition, SearchTree uses true agglomerative hierarchical clustering (AHC: Day & Edelsbrunner
411 (1984)) based on the BLAST estimates of sequence dissimilarity rather than the NCBI tree".

412 • a recent review on the state of large phylogeny (namely insects) generation using tools of the
413 data-driven era (Chesters 2019) cites phylota as a tool for homology inference and retrieval.

414 • the study presenting phylotol (Cerón-Romero *et al.* 2019), cites phylota as a tool that "focus on
415 the identification and collection of homologous genes from public databases".

416 • The iPTOL project cites phylota as a resource of phylogenetic trees.

417 • Mahmood (2015) PhD dissertation presents a database of avian Raptor sequences (raptorbase),
418 based on the phylota pipeline.

419 • Ruiz-Sanchez *et al.* (2019) develops datataxa and cite phylota as "software that has been developed
420 to mine the massive amount of information stored in GenBank", along with its R version (phylotaR;
421 Bennett *et al.* 2018) and restez https://www.rdocumen-tation.org/packages/restez/versions/1.0.0.

422 • The phylotastic project (Stoltzfus *et al.* 2013) cites phylota as a "phylogeny-related resource
423 providing ways to generate custom species trees *de novo* for downstream use" along with CIPRES.

424 2. When the software was actually used to construct (partially or in full) a DNA data set to be used for
425 phylogenetic reconstruction:

426 • A 1000 tip phylogeny of the family of the nightshades (Särkinen *et al.* 2013)

427 • A 56 tip phylogeny of crustacean zooplancton (Helmus *et al.* 2010) – ecological study

428 • A 63 tip phylogeny of the Salmonidae family (Crête-Lafrenière *et al.* 2012)

429 • A 321 tip phylogeny of Testudines (Thomson & Shaffer 2010)

430 • A 69 taxa phylogeny of the family Cyprinodontidae of the pupfish (Martin & Wainwright 2011)

431 • A 2,957 taxa phylogeny of the class Moniloformopses of living ferns (Lehtonen 2011)

432 • A 2,573 species phylogeny of the Papilionoidea (Hardy & Otto 2014)

433 • A 23 taxa phylogeny of the California flora (Anacker *et al.* 2011)

434 • Phylogenies of 6 different clades of flowering plants representing an independent evolutionary
435 origin of extrafloral nectaries: *Byttneria* (Malvaceae), *Pleopeltis* (Polypodiaceae), *Polygoneae*

(Polygoneaceae), *Senna* (Fabaceae), *Turnera* (Passifloraceae), and *Viburnum* (Adoxaceae) (Weber & Agrawal 2014).

- To supplement DNA data sets of various pre-existing mammalian phylogenetic trees sampled at different taxonomic levels (Faurby & Svenning 2015)
- A 900 species tree of muroid rodents, Muroidea (Steppan & Schenk 2017), where 300 species were newly added by the study and the rest obtained using phylota.
- A 95 taxa phylogeny of Gymnosperms, focused on Ephedra, Gnetales (Ickert-Bond *et al.* 2009)
- A 1061 genera phylogeny of the Oscine birds (Selvatti *et al.* 2015)
- A 268 species phylogeny of sharks, representing all 8 orders and 32 families (Sorenson 2014; Sorenson *et al.* 2014)
- A 466 species phylogeny of the Proteaceae, focusing on the species found in the Cape Floristic Region (Tucker *et al.* 2012).
- A series of small phylogenies of unreported exact size, of sister groups of gall-forming insects (Hardy & Cook 2010).
- A 196 species phylogeny of the family Boraginaceae (Nazaire & Hufford 2012). The authors actually found data for 318 Boraginaceae spp using phylota, but decided to reduce their data set to focus on the monophyly of genus *Mertensia*.
- A phylogeny of 401 species of scale insects Coccoidea, Hemiptera (Ross *et al.* 2013), with some sequences generated *de novo*.
- Two phylogenies sampling all species of two different clades of insectivorous lizards, agamids and diplodactyline geckos, groups considered to be radiating in the Australia's Great Victoria Desert (Rabosky *et al.* 2011)
- A phylogeny of 91 species of sparid and centracanthid fishes, Sparidae, Percomorpha, plus 2 outgroups, a lethrinid and a nemipterid exemplar (Santini *et al.* 2014).
- Updating a phylogeny of Arecaceae, constructing relationships in 6 cldes within the group: subfamilies Calamoideae and Coryphoideae, the tribe Ceroxyleae within subfamily Ceroxyloideae and three groups within subfamily Arecoideae: (1) Iriarteeae,

(2) Cocoseae: Attaleinae except Beccariophoenix and (3) a group containing six tribes; Euterpeae, Leopoldinieae, Pelagodoxeae, Manicarieae, Geonomateae and Areceae (Faurby *et al.* 2016).

- A phylogeny of 768 Gesneriaceae species and 58 outgroups for a total species sampling of 826 taxa (Roalson & Roberts 2016) some sequence were generated *de novo*.
- A phylogeny of 47 species of scombrid fishes, with 2 outgroups, a gempylid and a trichiurid (Santini & Sorenson 2013).
- to update a dataset underlying a large-scale fern phylogeny (Lehtonen *et al.* 2017), data set in https://zenodo.org/record/345670#.Xr9QFRPYqqg, also in TreeBASE, but it is one of those studies that is broken.
- A phylogeny of 13 species of billfishes, order Istiophoriformes: Acanthomorpha, and four outgroups (Santini & Sorenson 2013)
- A phylogeny of 765 aphid species, family Aphididae (Hardy *et al.* 2015)
- A phylogeny of less than 100 taxa of the family Ranunculaceae (Lehtonen *et al.* 2016), even though they retrieved info from phylota for 194 taxa within the family, they reduced their data set because of low sampling of markers for some taxa.
- A phylogeny of 144 neobatrachian genera, assuming the monophyletic status of genera to increase matrix-filling levels (Frazao *et al.* 2015).
- A 179 species phylogeny of the bird family Picidae (woodpeckers, piculets, and wrynecks) (Dufort 2015, 2016), augmented with data from an updated GenBank release and newly sequenced data.
- A phylogeny of species of freshwater fish endemic to NorthAmerica (Strecker & Olden 2014), phylota found data for 54 out of 66 spp.
- A phylogeny of 520 species of the order Ericales (Hardy & Cook 2012)
- A phylgeny of 16 fish species of the family Sphyraenidae (Percomorpha), as well as two outgroup species of the Centropomidae (barracudas) (Santini *et al.* 2015)
- A phylogeny of 34 vole species, Arvicolinae, Rodentia (García-Navas *et al.* 2016)
- Kolmann *et al.* (2017) uses phylota to download all 1691 co1 sequences belonging to the order Carchariniformes, to place phylogenetically DNA samples obtained from fish markets.

- A phylogeny of 329 bird species in the Tyrannidae (77% of the species in the family) (Gómez Bahamón & others 2015; Gómez-Bahamón *et al.* 2020)
- Retrive 145 sequences registered as *Holothuria* species, but kept 84 as ingroup, plus 4 outgroup sequences from *Stichopus ocellatus*, all belonging to the order Apodida of sea cucumbers (Kamarudin *et al.* 2016)
- On a master thesis, to get the sequences of the outgroups of Melinidinae, family Poaceae, namely several spp of the subfamily Panicoideae, plus *Gynerium sagittatum*, *Chasmanthium latifolium*, and *Zea mays*, (Salariato 2010). Interestingly, phylota was not used in the published study of the thesis (Salariato *et al.* 2010). Ingroup sequences were generated *de novo*.
- On a PhD thesis, to construct a phylogeny of Platyrrhini (internal group), Catarrhini (outgroup), and Tarsiiformes Pereira (2013). Have not found a published study.
- The 10k trees project (Arnold *et al.* 2010) uses phylota to construct a tree of 301 primate species and the outgroup species *Galeopterus variegates*, a tree of 17 extant odd-toed ungulates species and the outgroup species *Bos taurus*, and a tree of 70 different species of carnivorans and *Equus caballus* as outgroup. However, the do not cite it on the paper, but only on their documentation http://www.academia.edu/download/49690788/10kTrees_Documentation.pdf.
- Freyman (2015, also in 2017), use phylota to construct a phylogeny (or maybe only mine genbank???) of the Onagraceae and Lythracea, and compare it to the tool they propose, SUMAC.
- Blackmon (2017) PhD study applies phylota to reconstruct a 822 mite species tree.
- A study of the effect of poliploidy on niche evolution (Baniaga *et al.* 2018), uses phylota to get a DNA data set for 132 unique taxa of vascular plants from 16 families and 25 genera,and a tree of 33 genera from 20 different families comprising 1706 taxa.

3. When the website was used to identify sequences and markers available in GenBank for a particular group. In this cases, the dataset mining was either performed with other tools, or not performed at all and just used for discussion:
   - A 812 tips phylogeny of the Order Chiroptera (Shi & Rabosky 2015) – dataset constructed with PHLAWD

- A 1276 tips phylogeny of the Fabaceae (Group *et al.* 2013) – dataset constructed by hand (I think??)
- A review of dated phylogenies of fire-prone tropical savanna species from Brazil (Simon & Pennington 2012) – just for discussion of the lack of markers available for these species on GenBank
- A review of the phylogeetic sof the Apicomplexa, a parasitic phylum on unicellular protists (Morrison 2009).
- Three data sets from phylota (the suborder Pleurodira of side-necked turtles; the family Cactaceae of cacti; and the Amorpheae, a clade of legumes) were used to demonstrate and exemplify phylogenetic decisiveness (Sanderson *et al.* 2010)
- Mentioned in a PHD thesis (Gagnon & others 2016), but not on the final publication (Gagnon *et al.* 2016), phylota was used to state that there are very few sequences available for the Legumes (7,482 out of 19,500 spp) on GenBank's release 194 (Feb2013).

4. Sometimes, it was cited by mistake:
   - In this 630 tip phylogeny of the Caryophyllaceae study (Greenberg & Donoghue 2011) it might have been originally cited as an example of large phylogenies that reflect well supported relationships from previous smaller phylogenies. However, it was removed from the text but not from the final list of references. The DNA data set was constructed by hand most probably.
   - a study reconstructing the insect tree of life with 49,358 species, 13,865 genera, and 760 families within the order Insecta (Chesters 2017), uses its own algorithm (SOPHI) to mine public DNA databases (Chesters & Zhu 2014). It does not cite phylota as it should, but includes it in their references.
5. When phylota was used to extract full trees (not only DNA data sets or markers):
   - Page (2013) uses it to generate phylogenies for the bionames website, a "database linking taxonomic names to their original descriptions, to taxa, and to phylogenies" generated with phylota.
   - Deepak *et al.* (2013) uses a sample of phylota trees to test their method to remove conflict from MUL-trees (short for multi-labeled trees), that is, phylogenetic trees with two or more leaves sharing a label, e.g., a species name, which can imply multiple conflicting phylogenetic relationships

for the same set of taxa.

- A review by Sanderson *et al.* (2016), takes 134 595 gene trees from phylota GenBank rel. 176 and estimates its degree of resolutin, calculating that less than half of clades are supported with minilam statistical support (0.53 ± 0.32).

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8 Authors' Contributions

9 Data Avilability

10 References

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