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Fachbereich Mathematik und Informatik

**An analysis of maximum parsimony algorithms to predict
parasitism in Eukaryota**

using a large multifurcated phylogenetic synthesis tree

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Abstract

Parasitism can be defined as an interaction between species in which one of the interaction partners, the parasite, lives in or on the other, the host. The parasite draws food from its host and harms it in the process. According to estimates, above 40% of all eukaryotes are parasites. Nevertheless, it is computationally difficult to obtain information whether a particular taxon is a parasite making it difficult to query large sets of taxa.

Here we test in how far it is possible to use the Open Tree of Life (OTL), a synthesis of phylogenetic trees on a backbone taxonomy (resulting in unresolved nodes), to expand available information via phylogenetic trait prediction. We use the Global Biotic Interactions (GloBI) database to categorise 25,962 and 34,860 species as parasites and free-living, respectively and predict states for over ~ 2.3 million (97.34%) leaf nodes without state information.

We estimate the accuracy of our maximum parsimony based predictions using cross-validation and simulation at 60-80% overall, while strongly varying between clades. The cross-validation results in an accuracy of 98.17% which is explained by the fact that the data are not uniformly distributed. We describe this variation across taxa as associated with available state and topology information. We compare our results with several smaller scale studies, which used manual expert curation and conclude that computationally inferred state changes largely agree in number and placement with those. In clades in which available state information is biased (mostly towards parasites, e.g. in Nematodes) phylogenetic prediction is bound to provide results contradicting conventional wisdom.

This represents, to our knowledge, the first comprehensive computational reconstruction of the emergence of parasitism in eukaryotes. We argue that such an approach is necessary to allow further incorporation of parasitism as an important trait in species interaction databases and in individual studies on eukaryotes e.g. in the microbiome.

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1 Introduction

This thesis is about the analysis of ancestral state reconstruction algorithms for non-binary trees, applied to the currently largest phylogenetic synthesis tree of Open Tree of Life (OTL) [1] to predict parasitism in Eukaryota.

For about 50 years, people have been working on ancestral state reconstruction, the inference of evolution that leads to the given data.

One of the first papers was written by Camin and Sokal, who in 1965 were working on algorithms for discrete-state data [2]. Different methods have been developed and the question is which method is the most suitable for the problem at hand: the ancestral state reconstruction for a huge non-binary tree with two discrete states.

Royer-Carenzi et al. distinguish two major classes of ancestral state reconstruction methods: The first is maximum parsimony: explain the current state with the least number of state changes between the child and its ancestor.

The other class they present describes modeling the character evolution as a stochastic process and using the likelihoods to compute the possible ancestral character states. This is generally done with a continuous time Markov model [3].

TODO: Pasqualin et al. unterscheiden noch eine weitere Methode: stochastic mapping...

One of the major disadvantages of parsimony methods is that, unlike likelihood approaches, they can not take divergence times (branch length) into account. Since the OTL does not include development times of species, this can not be considered here.

Another problem pointed out by Royer-Carenzi is that parsimony approaches are either based on predefined parameters (generalized parsimony) or on strong and often controversial assumptions, like irreversibility of transitions for dollo parsimony. This problem is unimportant to the present task because in the analysis of the entire Eukaryota tree only generalized

models are meaningful.

The comparison of methods gives us the maximum parsimony method as the simplest and fastest method that meets all our requirements.

Felsenstein [4] discusses in his book two parsimony algorithms that generalize previous methods (from Camin and Sokal [2], Farris [5] and others): Fitch parsimony [6] and Sankoff parsimony [7]. Therefore, these are the methods used in this work.

For Fitch, the algorithm has been extended from binary to non-binary trees. For the Sankoff algorithm, Louca and Doebeli have presented an implementation for non-binary trees published in an R package named *castor* [8].

For an ancestral reconstruction on the phylogeny of Eukaryota, we use Open Tree of Life (OTL) [1].

Phylogeny describes the evolution of species, while the taxonomy is a classification according to certain criteria in so-called taxa.

OTL is a comprehensive, dynamic and digitally available tree of life constructed from published phylogenetic trees along with a backbone of taxonomic data. Besides this, Hinchliff et al. also offer an OpenTreeOfLife-Taxonomy (OTT) with the help of which we identify the individual nodes.

It follows that the biggest 'phylogenetic tree' is this synthesis of phylogenetic trees filled with a taxonomic trees given by OTL.

For these large phylogenetic synthesis trees, however, ancestral state reconstruction has so far only been done for Bacteria and Archaea for binary traits by Goberna and Verdú [9]. However, this differs from eukaryotes in the sense that complex traits such as parasitism depend on more than one gene.

The present tree structure of OTL is not binary but multifurcated, meaning that each node has multiple ($n > 2$) children or in other words its degree, number of adjacent nodes, is greater than 3 [4].

Parsimonious in phylogeny refers to favoring the tree that needs the least evolutionary change to explain the observed data. Maximum parsimony methods have been developed for phylogenies, which are usually depicted as binary trees. Therefore, the selected parsimony methods are not directly applicable, as they were specifically applied to much smaller subtrees, where all splits are known.

We will extend and test the existing maximum parsimony algorithms of Fitch [6] and Sankoff [7] for this task and estimate their predictive power.

The Sankoff method is implemented by Louca et al. for the non-binary case and is available as an R package called *Castor* [8].

Note, the original Fitch algorithm has the sole purpose of minimizing the number of transitions and not reconstructing the ancestral nodes. Felsenstein [4] describes a simple extension for the reconstruction. In this work, the algorithm extended to reconstruction is adapted to multifurcated trees, based on the critical reevaluation of this extension by Cunningham et al. [10].

To accomplish this task, next to the phylogenetic tree information about the current species' states is needed.

Most of the largest interaction databases are offline or outdated. For example: IWDB (Interaction Web Database) [11], Webs on the Web [12], Animal Diversity Web [13] and ecoweb [14].

We use the interaction database Global Biotic Interactions (GloBI) [15] because it is including most of the known databases and is still growing actively [15].

The data in GloBI are stored as interactions e.g. species A parasitize species B. From this we conclude that species A is parasitic and species B free-living.

From this data, we could specify ~2.3 million leaf nodes 34,860 as free-living and 25,962 as parasitic.

There are many different ways to define parasitism. Since we use GloBI to classify species, we use their definition of parasitism. Again, in GloBi, Ontobee definitions are used [16].

The interaction *has parasite* is defined as: "An interaction relationship between two organisms living together in more or less intimate association in a relationship in which association is disadvantageous or destructive to one of the organisms."¹. This definition includes: ecto- and endoparasites, parasitoids, kleptoparasites and pathogenes.

The objectives of this work are the following points: (1) Find a suitable ancestral state reconstruction method. (2) Accomplish reconstructing on the Eucaryotic synthesis tree of OTL.

¹ontobee.org/ontology/RO?iri=http://purl.obolibrary.org/obo/RO_000244; Last checked: 22.03.2018.

The goal of Point 1 is to evaluate the possible methods based on a simulation of our data situation.

The Sankoff algorithm implemented by Louca et al. is the best in our comparisons. Therefore, point 2 consists of reconstructing the ancestral states and predicting the unknown leaf states with the aid of this algorithm. And then perform an evaluation of the results.

2 Aims

The aim of this thesis is the application of maximum parsimony algorithms to non-binary trees and very large data sets to predict unknown leaf node states.

We discuss different ancestral state reconstruction methods and test the most appropriate ones on our data. Hereby, we want to find out how far it is possible to use the Open Tree of Life (OTL) to expand available information via phylogenetic trait prediction. As a basis for known states, we use the Global Biotic Interactions (GloBI) database, which categorises 34,860 and 25,962 species as free-living and parasites. This data is used to perform an ancestral state reconstruction and predict states for over ~ 2.3 million (97.34%) leaf nodes without state information.

In order to generate a realistic simulation, influencing parameters are investigated.

Since the transitions are minimized in an ancestral state reconstruction, this is an important parameter to consider. On the other hand, the completeness of our input data is an influencing value. Therefore, two major types are distinguished:

i) Biological parameters (a result of the evolutionary process):

- State distributions and transition probabilities

ii) Distribution of missing information:

- Lack of information on topology (\rightarrow multifurcations)
- Lack of information of states of some leaf nodes

3 Methods

In this thesis, a maximum parsimony algorithm is applied to the Eukaryota tree to obtain an ancestral state reconstruction of free-living versus parasite states.

This chapter is divided into the following sections: the description of the data sources (section 3.1) used and the analysis of these data as a preparation for the simulation (section 3.2). A description of the analyzed methods for the ancestral state reconstruction (section 3.3) and then an explanation of how they fit the problem at hand (section 3.4). And at the end, the real data analysis with the Sankoff method (section 3.5).

Figure 3.1 briefly outlines these relationships. A more detailed view of the workflow can be found in the appendix 7.1.

3.1 Data sources

Two types of data are needed for an ancestral state reconstruction: a tree and information about the states.

For the subsequent analyzes on the Eukaryote tree the database of Open Tree of Life (OTL) is used (downloaded on 16.02.2018) [1]. This database gives a synthesis of phylogenetic trees (currently 819 trees) and a taxonomic tree¹. OTL also includes the large phylogenetic database TreeBASE [1].

Furthermore the Open Tree Taxonomy (OTT) from OTL is used because it includes most of the known taxonomies and is synthesised by preferring taxonomies that match with available phylogenetic data. The team from OTL prefer a maximum number of species [1], this results in the synthesis between taxonomy and phylogeny.

¹<https://tree.opentreeoflife.org/about/synthesis-release/v9.1>; Last checked: 22.03.2018.



Figure 3.1: The Workflow of the resulting procedure with the following steps:

- (1) Retrieve phylogenetic tree data as input for the tree (OTL) and the state information (GloBI).
- (2) Get metadata of these for a realistic simulation of the maximum parsimony algorithms (Fitch & Sankoff).
- (3) Build and run the simulation.
- (4) Evaluation of parameters for the simulation and the ancestral state reconstruction of the real tree.
- (5) Evaluate the accuracy of developed algorithms and choose the best.
- (6) Run the resulting algorithm on the original data.
- (7) Evaluate and interpret the results.

For the state information the Global Biotic Interactions database (GloBI) is taken [15] (downloaded on 29.01.2018).

This database consists of entries of the form: species A (source) interacts with B (target). A number of interactions have been identified², including those indicating whether the species source or target has become a parasite or a free-living species from the biological perspective. They are the following:

- free-living source: preysOn, eats, flowersVisitedBy, hasPathogen, pollinatedBy, hasParasite, hostOf
- free-living target: preyedUponBy, parasiteOf, visitsFlowersOf, pathogenOf, hasHost
- parasite source: parasiteOf, pathogenOf

²<https://github.com/jhpoelen/eol-globi-data/.../InteractType.java>; Last checked: 22.03.2018.

- parasite target: hasParasite, hasPathogen

Of these interactions, e.g. species A parasitize species B, the state of the species is determined, here is species A parasitic and species B free-living. In the case that a parasite conquers (parasitizes) another parasite yields conflicting states for the second species. This is solved by preferring parasitic.

For each species known identifiers are stored in GloBI. This includes OTT (the taxonomy of OTL). All species that have stored an OTT identifier and have a matching interaction are divided into two lists: parasites and free-livings.

3.2 Metadata analysis

Based on the input data, generalized linear models are compared with poisson respectively binomial regression according to their residuals. In order to compare models of different complexity, the BIC (Bayesian Information Criterion) values are calculated in addition to the residuals.

There are two different information criteria: AIC (Akaike Information Criteria) and BIC.

The advantage of the BIC is that the penalty is dependent on the sample size and is therefore advantageous for large samples.

For all these calculations, the following R functions are used: *glm()*, *anova()* and *BIC()*.

For each node, depth, min, max and mean height are noted. The node depth is calculated as the distance (number of edges) to the root node. The node height is calculated in three different ways: min, max and mean height refers to the (minimal, maximal, mean) distance to a leaf node.

The influence in the modeling of these parameters is tested, additively as well as multiplicatively to the models.

3.3 Ancestral state reconstruction methods

The methods used to reconstruct the ancestral states are Fitch parsimony [6] and Sankoff parsimony [7], which are explained in the following subsections.

3.3.1 Fitch maximum parsimony

Based on the work of Cunningham et al. [10] the Fitch method [6] is implemented and extended by us for the multifurcated tree case. To understand the differences to the multifurcated case, the algorithm for the binary case is briefly explained and referred to the extension.

Input: A rooted, binary tree, with state informations in the leaf nodes. Each node is depicted as a set of states. There are only two states in this thesis, free-living (FL) and parasitic (P). Internal nodes have three sets, which are empty at the beginning, excluding the root node, it has only one. Leaf nodes have their state as a set (e.g. {FL} or {P}, unknown leaf nodes the union of all possible states ({FL, P})).

The algorithm traverses three times through the tree and fills these sets.

In each step, two sets are considered and their intersection is formed. There are two cases:

- i) The intersection is not empty and corresponds to the new set.
- ii) The intersection is empty. → Build the union of these sets as new set.

First traverse from the leaf nodes to the root: each internal node is formed from its child nodes, where the only information lies at the beginning.

Second traverse from the root node to the leafs: each internal node is formed from its parent node and its sibling node.

Last traversal (direction does not matter): build the final state for every node. It is formed from the sets of previous traversals.

(The original Fitch algorithm is designed to minimize transitions without predicting actual states of internal nodes, so it is just the first traversal.)

The extension to the non-binary case is quite obvious, but holds some opportunities. In this case, more than two children may be present for the first traversal, but the incision or union may also be formed over more than two sets. Also in the second traversing, there may be several sibling nodes. However, there are several possibilities here that are all tested and compared in the simulation. Some of these options are already available in the binary case:

- The parent node has (except for the root node) two state sets, because he came through the up-traversing previously. Are both sets used or only the first traversing?
- If there are several siblings, is the cut or union made first of these, or is everything made directly including the parent node.

The first point already has an effect on the binary case. Figure 3.2 shows both possibilities of the three sets.

Cunningham uses only the first state set of the parent node [10].

From these two points four different versions of Fitch are formed:

- Fitch 1: First state set of parent node; intersection/union of siblings first.
- Fitch 2: First state set of parent node; intersection/union of siblings together with parent node.
- Fitch 3: Both state sets of parent node; intersection/union of siblings first.

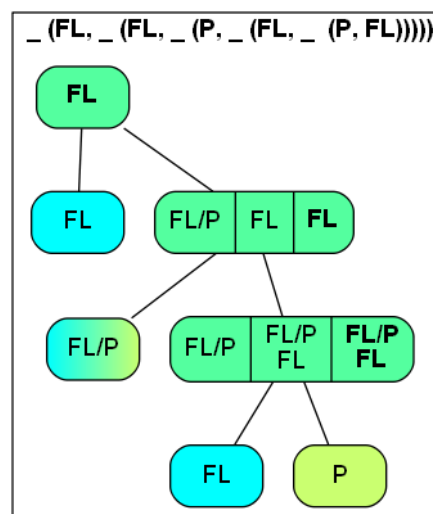


Figure 3.2: Fitch algorithm for binary trees.

The unknown leaf node is discribed with both states. Computed internal nodes (exclusive the root node) consists of three sets, where the last set is the final one (bold).

From the second internal node (seen from the root node) there are several possibilities to create the second and third set.

- iv) Fitch 4: Both state sets of parent node; intersection/union of siblings together with parent node sets.

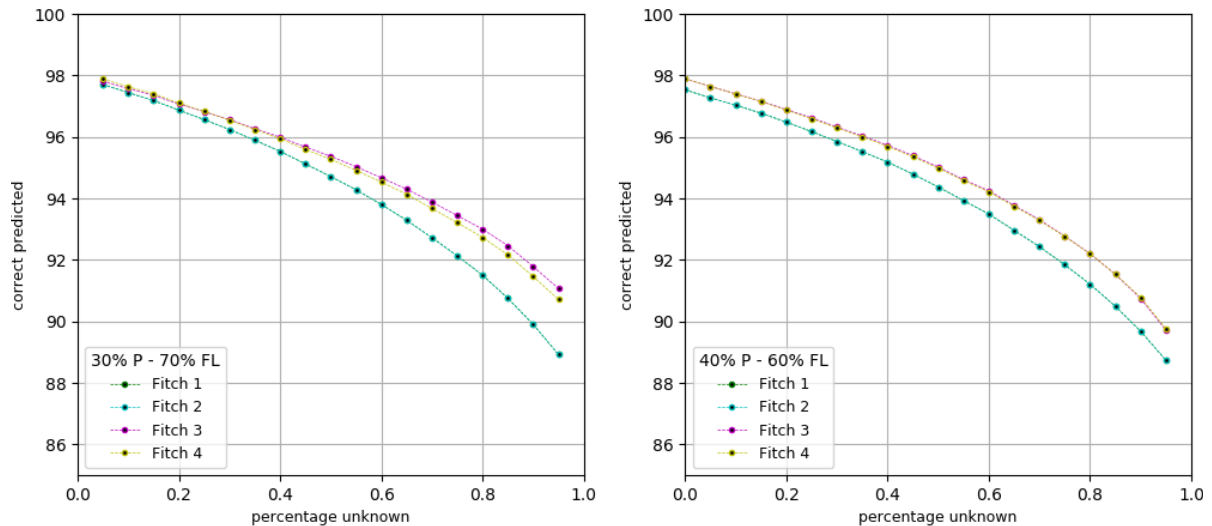


Figure 3.3: Test of Fitch Versions. TODO: beschriften!!

These four versions are tested in the simulation with 100 trees and 10000 leaf nodes and distributions of 70% FL to 30% P and 60% FL to 40% P. Figure 3.3 shows this over all unknown node percentage.

At 95% unknown nodes and 95% of multifurcation of the internal nodes, version 1 is 88.37%, version 2 is 88.37%, version 3 is 88.4%, and version 4 is 88.39% correct. Therefore, only version 3 is used for all further simulations.

Sankoff

Maximum parsimony algorithm from Sankoff implemented in the R package *castor* [8]. From this the function `hsp_max_parsimony()` is used with default settings including `transition_costs = "all_equal"`.

3.4 Simulation

The simulation compares these different ancestral state reconstruction algorithms with each other.

First different implementations of the Fitch maximum parsimony are compared and then the best of them is compared with the implementation of the Sankoff algorithm of the *Castor* package [8].

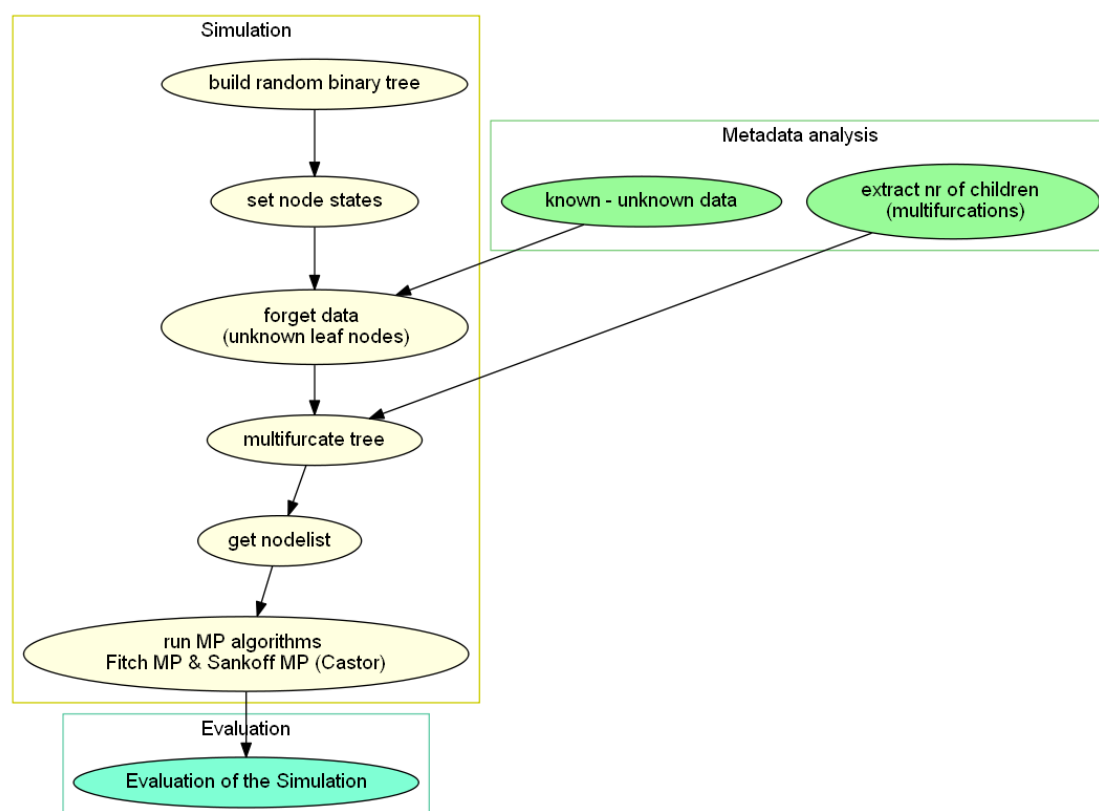


Figure 3.4: A simulation is performed to compare different maximum parsimony algorithms. The course of the simulation with influence of the metadata analysis from the real data can be seen:

- (1) A phylogenetic tree is created randomly.
- (2) Simulate node states for all nodes.
- (3) 'Forget' internal states and some leaf node states.
- (4) 'Lose' phylogeny information.
- (5) Make a nodelist for the algorithm.
- (6) Run algorithms.
- (7) Evaluate results.

Points 3 and 4 are influenced by metadata of the real-data analysis.

The course of a simulation is shown in Figure 3.4. The individual steps are explained in the following.

A tree is needed to perform a simulation of ancestral state reconstruction. It has to be decided whether to take the real tree or simulate a tree. In this simulation, trees are created randomly, as one can replicate a complete binary phylogentic tree. Thus, there is also the possibility to simulate the multifurcation.

To get a random binary tree, the Phylo package from biopython is used [17]. They offer a *randomized()* function which returns a BaseTree³.

The next step is to simulate states for all nodes.

Here the influence of the biological parameters as transition probabilities and distributions of states is included.

Since there is no statement about general transition probabilities, these are all set the same:

$$\mathcal{P}(FL \rightarrow P) = \mathcal{P}(P \rightarrow FL).$$

For the distributions of states different ratios of parasites (P) to free-livings (FL) are simulated with the help of beta distributions and a given threshold:

- 50% P to 50% FL,
- 40% P to 60% FL,
- 30% P to 70% FL and
- 20% P to 80% FL.

Procedure: The root node is defined as ancestor of all subsequent species and in this case, determined to be free-living. Therefore, the beta distribution for free-living is used at the beginning. Now traverse from the root to the leaf nodes, always pulling out of the current

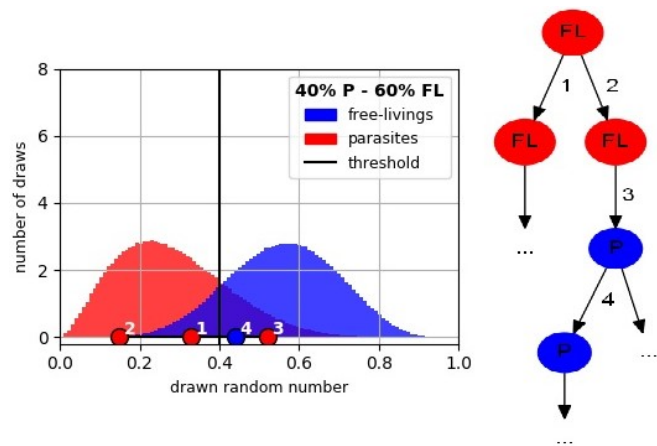


Figure 3.5: Set node states: Distribution of states (left); traversal through the tree (right). Start with a free-living root node (FL: red).

(1) + (2) Draw random numbers for its children from the free-living distribution (red), the numbers are under the threshold → the nodes are again free-living; go on with the children.

(3) The number drawn is above the threshold. → The node state changes to parasitic (P: blue).

(4) Now draw random numbers from the parasite distribution (red) until one number lies under the threshold. Then change back.

³<https://github.com/biopython/biopython/blob/master/Bio/Phylo/BaseTree.py>

distribution until the randomly drawn number is above the threshold and the new node changes state. Figure 3.5 shows a part of this simulating states and the associated distributions.

After traversing through the tree, each state is saved in a nodelist associated with the node identifier which is the OTT from OTL.

Here begins the simulation of the lack of information, such as the lack of information on topology (multifurcation) and of states of some leaf nodes.

In the real tree, there is usually only information about species living today → leaf nodes. And beyond only a small percentage of these. All information about the states of the internal node and one leaf node is removed and stored in another column to the node.

Last step for the preparation is the multifurcation of the tree. As previously explained, some divisions in the tree are not known, so the real tree is not binary. This multifurcation is simulated by an equally distributed percentage of forgotten internal nodes.

Different percentages of removed information are simulated.

The last step is the evaluation of the results. This is done with a simple difference calculation of the node states.

In the nodelist, the originally simulated states and the newly calculated states are stored for each node ($FL = 0$, $P = 1$). The sum of the differences of the node states gives the distance of the prediction to the original tree.

3.5 Real data analysis

For the evaluation of real data results, a leave-100-out cross-validation is performed. For this purpose 100 randomly distributed 100 states are left out.

Analogous to the missing leaf node states, generalized linear models with binomial regression are compared according to their residuals, based on the "true" / "false" prediction.

Again, in order to compare models of different complexity, the BIC (Bayesian Information

Criterion) values are calculated in addition to the residuals.

For all these calculations, the following R functions are used: *glm()*, *anova()* and *BIC()*.

3.6 Implementation

The complete code is located on GitHub: github.com/Irallia/IZW-HU-Parasites.

Most of the code is written in Python, the analyzes and the use of the Castor package in R.

Some shell scripts are used to execute whole workflows.

4 Results

This work deals with the ancestral state reconstruction of the entire Eukaryota relatives tree.

For this reconstruction, we first analyzed our data. This is the tree of OTL [1] and the data for the leaf node states (free-living or parasite states) from GloBI [15] (section 4.1), where we have determined that the entire Eukaryota tree is 89.45% multifurcated (missing internal nodes) and that we do not have any state information for 97.34% nodes.

In addition, we tested different models for these two parameters and found out that multifurcation can best be modeled with the taxa 'order' multiplied by the mean height of the knots. Also, the missing states could be modeled with the taxa 'order' and in this case multiplied by the depth of the nodes.

Next we compared different possible methods. We decided to take a closer look at maximum parsimony algorithms because they are fast and best suited to the problem at hand. We tested these on different simulations of the data and compared their predictive power (section 4.2). We compared them with the result that the Sankoff algorithm performs significantly better than Fitch. The multifurcation rate has little impact on Sankoff, but Fitch's prediction significantly breaks down at more than 60% missing internal nodes. However, the amount of missing node state information has a linear impact on both, as does the ratio of free-living to parasitic (the more balanced, the worse is the prediction).

As a result, we used the Sankoff algorithm [7] implemented by Louca et al. [8], which performed best, for the actual reconstruction on the real data.

TODO: ... biological view We evaluated these by performing a leave-100-out cross-validation and predicted approximately 98.17% of the omitted states correct (subsection 4.3.2).

4.1 Availability of internal nodes and state information

As previously presented, we have two types of missing information: unknown states of leaf nodes and multifurcation.

A tree is multifurcated if there are nodes that have more than two children. A binary tree with n leaf nodes has $n - 1$ internal nodes. The present Eukaryota tree of OTL has 2,293,463 leaf nodes and only 241,974 internal nodes, that is:

$$100 - \frac{100}{(2293463 - 1) \times 241974} \approx 89.45\%$$

missing internal nodes. This means that there is a lack of information about the underlying phylogeny. Instead of a binary tree this tree is highly multifurcated.

| Subtree of | Unknown States | Multifurcation |
|-----------------|----------------|----------------|
| Eukaryota | 97.34% | 89.45% |
| Metazoa | 96.44% | 87.93% |
| Fungi | 98.87% | 96.97% |
| Chloroplastida | 99.14% | 89.46% |
| Apicomplexa | 86.26% | 87.16% |
| Nematoda | 89.01% | 88.59% |
| Chordata | 88.59% | 66.49% |
| Platyhelminthes | 68.73% | 80.34% |
| Insecta | 97.11% | 90.78% |

Table 4.1: Examination of subtrees regarding missing information.

The percentage values show the proportion of missing information of: unknown states (missing state information of leaf nodes) and multifurcation (missing internal nodes). The subtrees are from different taxa: domain (Eukaryota), kingdom (Metazoa, Fungi, Chloroplastida), phylum (Apicomplexa, Nematoda, Chordata, Platyhelminthes) and class (Insecta).

The two by far smallest values are highlighted in green.

For the missing state information the present Eukaryota tree with 2,293,463 leaf nodes,

34,869 free-livings and 25,962 parasites are found. This gives

$$100 - \frac{100}{2293463 \times (34860 + 25962)} \approx 97.34\%$$

unknown states of leaf nodes.

We calculated these percentages of missing nodes and also missing state information for some subtrees and plotted them in table 4.1.

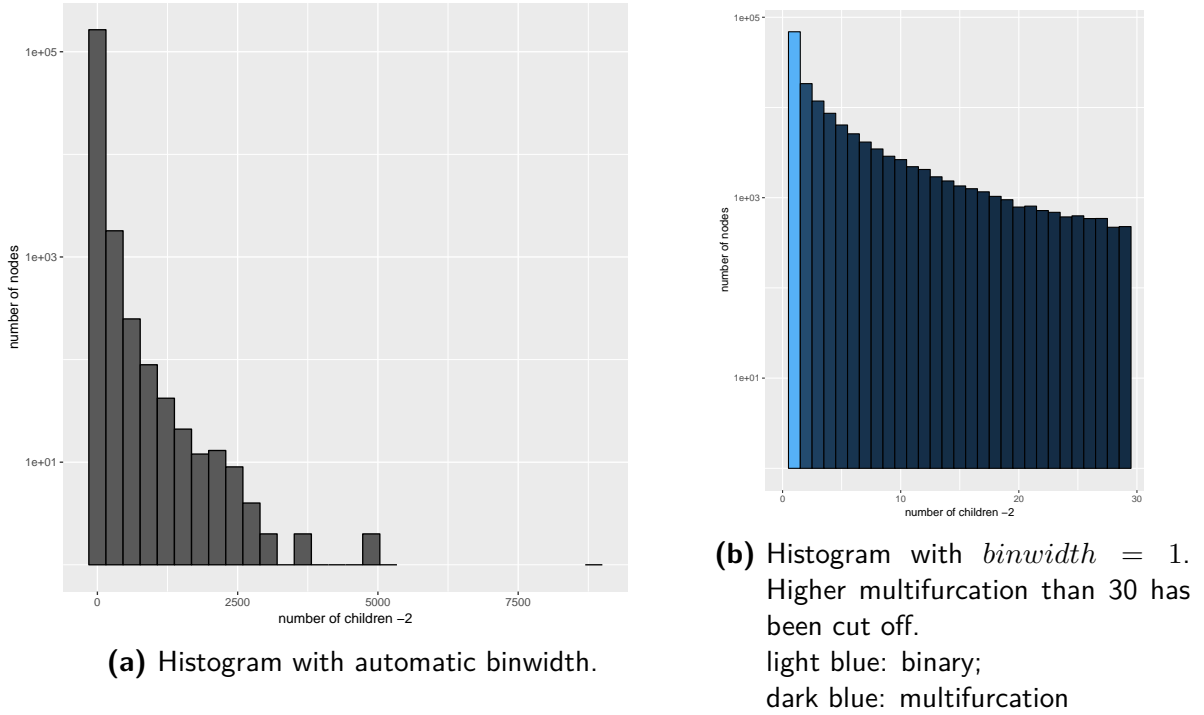


Figure 4.1: Histograms showing the multifurcation of the internal nodes of the synthesis tree. For each node, the number of children (degree -1) is collected. A node is multifurcated if it has more than two children, so we deducted two from each number of children. The two histograms show: number of children -2 on the x-axis with log scale and the number of nodes with this amount on the y-axis.

For a first overview, we collected for each node its number of children (degree -1), and plotted this in two histograms, see figure 4.1.

The multifurcation affects only the internal nodes. We collected the number of children -2 of these nodes (because a node with two children is binary). That means it describes the

number of nodes which we have lost from the real (binary) phylogenetic tree.
It can be recognized that we are very far from a binary tree.

4.1.1 Comparison of different models for multifurcation

For the regression analysis of the multifurcation we set up several generalized linear models that could describe multifurcation.

In doing so, we allowed the different influence of the taxa and the heights and depths of a node to be included. From this we got 9 times 4 models of different complexity levels (first row) and the associated BIC values (table 4.2).

| Model / Taxa | Kingdom | Phylum | Class | Order |
|--------------------------------|---------|---------|---------|---------|
| multifurc ~ taxa | 8273333 | 7937828 | 7842157 | 7644249 |
| multifurc ~ taxa | 8257680 | 7922207 | 7826490 | 7574154 |
| multifurc ~ taxa + depth | 8273318 | 7934322 | 7839364 | 7539999 |
| multifurc ~ taxa + max.height | 7993515 | 7749121 | 7661817 | 7416211 |
| multifurc ~ taxa + min.height | 8251211 | 7875521 | 7778327 | 7516883 |
| multifurc ~ taxa + mean.height | 7825417 | 7644249 | 7572474 | 7340741 |
| multifurc ~ taxa * depth | 8235932 | 7836755 | 7757688 | 7383808 |
| multifurc ~ taxa * max.height | 7963438 | 7693555 | 7614820 | 7335338 |
| multifurc ~ taxa * min.height | 8214030 | 7808940 | 7690618 | 7336627 |
| multifurc ~ taxa * mean.height | 7768360 | 7536296 | 7484953 | 7206369 |

Table 4.2: BIC (Bayesian information criterion) values of the multifurcation models.
These models are created with the R function *glm()* and compared with the *BIC()* function. This results in the listed BIC values, presented in a heatmap colorization.

Within every complexity class it can be seen that the mean height gives the best additional factor.

Despite higher complexity, the BIC values are getting smaller from model to model, meaning that the finest model available here is also the best one of these. Lower taxa than orders (e.g. family) are computationally too expensive to calculate.

The model *multifurc ~ order * mean.height* turns out to be the best of our models, whereby it is possible that e.g. *multifurc ~ family * mean.height* is better.

4.1.2 Comparison of different models for missing state information

Next to the problem of the multifurcation of the tree is the little interaction data that we have for the species. For the ancestral state reconstruction, we need information about the states (free-living or parasite) in the leaf nodes.

The eukaryotic synthesis tree has 293,463 leaf nodes. The GloBI database has 5,346,414 interactions (at 29.01.2018). Out of this data we got 51,337 free-living species and 47,332 parasite species for the whole tree of life. From the Eukaryota we could determine 25,962 and 34,860 species as parasites and free-living. With 2,293,463 leaf nodes we still have about 97.34% unknown leaf nodes.

We also compared different models in terms of their BICs (Table: 4.3).

| Model / Taxa | Kingdom | Phylum | Class | Order |
|--------------------------|---------|--------|--------|--------|
| multifurc ~ taxa | 545799 | 500004 | 485121 | 484681 |
| multifurc ~ taxa + depth | 544862 | 493808 | 481869 | 478851 |
| multifurc ~ taxa * depth | 544179 | 489845 | 481494 | 478188 |

Table 4.3: BIC (Bayesian information criterion) values unknown state information models. These models are created with the R function *glm()* and compared with the *BIC()* function. This results in the listed BIC values, presented in a heatmap colorization.

It also follows from this table that the most complex model is the best. In general, the BIC values are smaller than those of the multifurcation models. The modeling here is thus better. Again, the calculation of finer models (e.g. family) was too expensive.

These missing data modeling results could be used to better simulate the data.

4.2 Influence of different parameters on the prediction

As presented, we compare two methods in our simulation to their prediction accuracy: Fitch and Sankoff.

First, we tested different distributions of parasites to free-livings including threshold (first column). It can be observed that the more balanced the percentages of free-livings and parasites, the worse the prediction of the algorithms (second and third column).

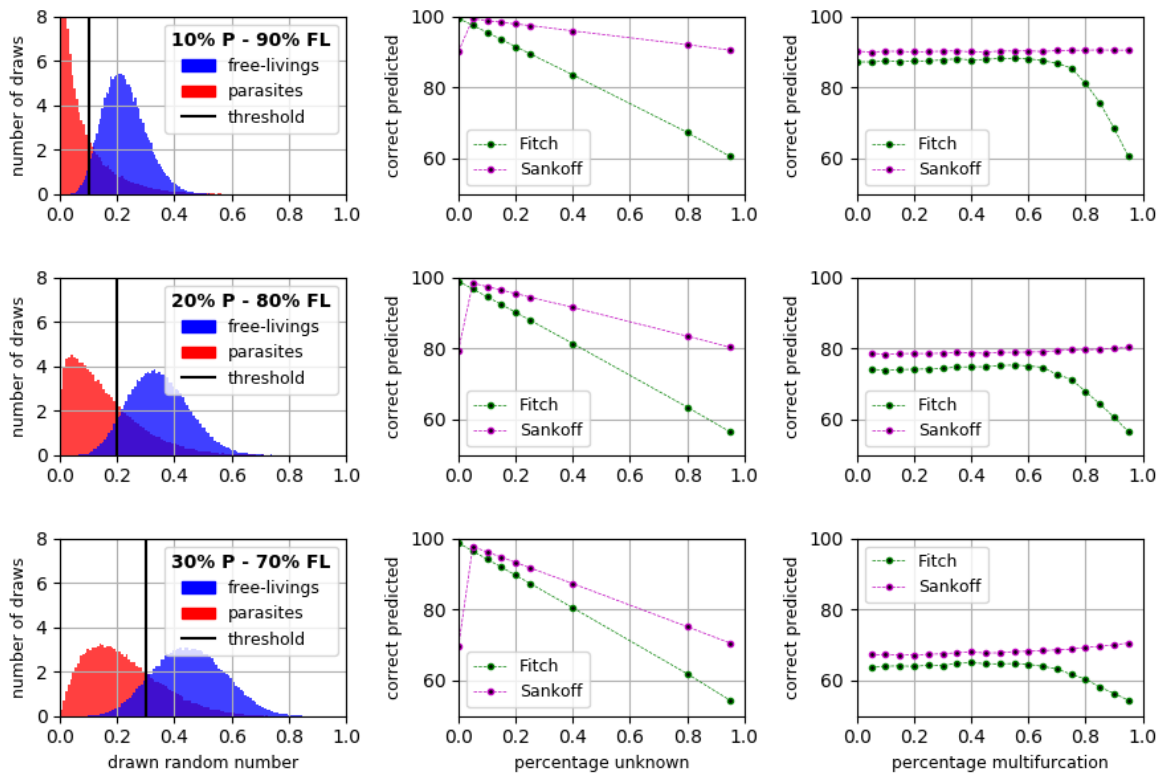
It can be seen that the predictive power of Sankoff is always greater or equal to the percentage of free-living distribution, and therefore more accurate than guessing.

On the other hand, we examined the influence of missing internal nodes (ridge of multifurcation) and missing leaf node information (unknown leaf nodes).

Both factors have a relatively linear influence on the Sankoff method. Fitch, on the other hand, breaks significantly in his prediction from about 70% unknown leaf nodes or multifurcation.

Figure 4.2 shows the results of examining various parameters.

Since we have a lot of missing data in most subtrees (both internal nodes and state informations), the Sankoff gives a better prediction and is thus used for real data analysis.



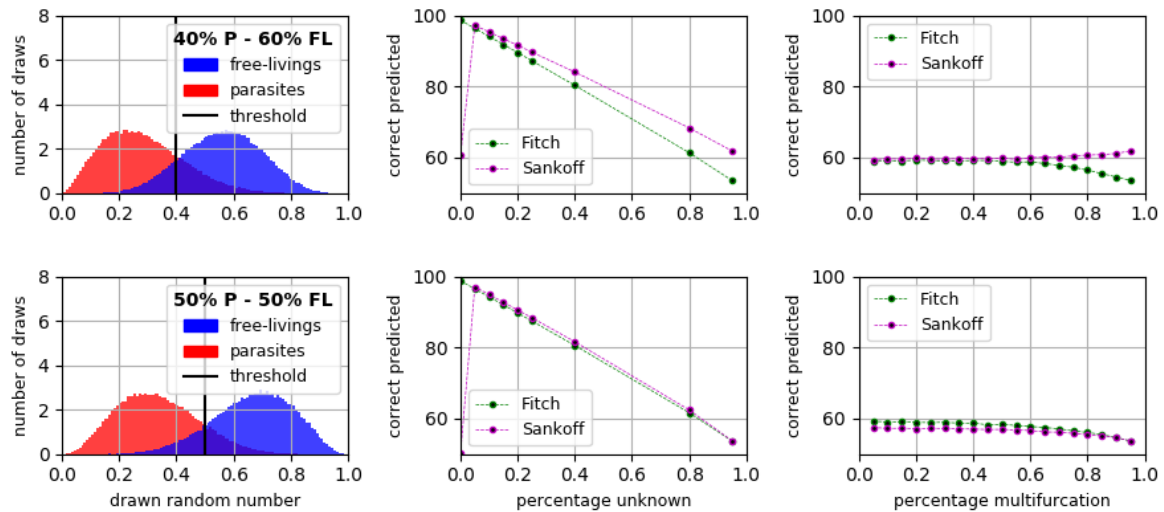


Figure 4.2: Influence of unknown data to prediction.

The first column describes the distributions of free-livings and parasites with a given threshold for the respective simulations to the right. Note, these distributions are chosen to form the specified proportions with the threshold, ignoring the number of state changes.

The middle column investigates the influence of the unknown states, the right the influence of the strength of the multifurcation.

The y-axes indicate the percentage of correctly predicted states (including known states). On the x-axis the percentage of forgotten states or missing internal nodes. Each point corresponds to the average of one hundred simulations, each with 10,000 leaf nodes.

For the middle column we set the strength of the multifurcations to 0.95%, similar to the real data and in the right column the amount of the unknowns to 0.95% also similar to the real data.

4.3 Results of the real data analysis created with Sankoff

This section is about evaluating the prediction of real data using the Sankoff method.

It is divided into two subsections. 4.3.1: The analysis of some subtrees using biologi-

cal background knowledge. 4.3.2: Presentation of the results of the leave-100-out cross-validation.

4.3.1 Biological view

To analyze the results, we have selected some phyla (subtrees) to evaluate our results selectively from the biological point of view: Chordata, Nematoda, Platyhelminthes and Apicomplexa.

In Table 4.4 we compare the given states with the predicted ones. Several factors such as the distribution of existing input data via parasitics and free-livings, faulty input data from GloBI and reinforcement of errors by multifurcation play an important role, which crystallize out in these examples. An important factor here is the credibility of the results. The accuracy of the input data stands and falls with the presence and the correctness of the data of GloBI. Errors of incorrect input data can be amplified by incorrect prediction of unknown species and can be reversed in order to improve the data situation of GloBI. Since we look at such large trees we can not expect to know all the parasites, so we look at individual positives. This is positive in the sense that the majority have the opposite state.

| | | | | | |
|------|------|------|------|------|------|
| | Text | Text | Text | Text | text |
| rota | text | | | | |
| | text | | | | |
| | text | | | | |

In contrast to the other phyla examined, the phylogeny in the Chordata is more pronounced (less multifurcation) (see Table 4.1). This results in less variance of errors. What is reflected in the results from the table 4.4. There are 18 parasites as input data and only 8 more are predicted. The Chordata mostly consist of free-living species, so this seems believable. We started with 99.83% species and predict 99.97% species as free-living (including already known nodes).

We mapped the few parasitic species in a rough/simple taxonomy (see Figure 4.3): Known parasitic birds belong to the order Sauria. Here we know from Rothschild here there

| | Taxa subtree | #internal nodes | #leaf | known states | | final states | | | Root node state | #origins (with rounding) FL → P | #losses P → FL |
|---------|----------------------|-----------------|---------|-----------------|-----------------|-------------------|----------------|------------------|-----------------|------------------------------------|-------------------|
| | | | | FL | P | [0, 0.5) FL | 0.5 | (0.5, 1] P | | | |
| Domain | Eukaryota | 241974 | 2293463 | 34860 57.31% | 25962 42.69% | 2053212 80.98% | 7775 0.31% | 474450 18.71% | 1 P | 462 | 369 |
| Kingdom | Chloro- plastida | 43486 | 416478 | 3519 97.86% | 77 2.14% | 410795 98.63% | 4182 1.00% | 1501 0.36% | 0.5 | 97 | 222 |
| | Fungi | 9534 | 31457 | 577 16.21% | 2983 83.79% | 38520 12.40% | 5723 1.84% | 266463 85.76% | 0 FL | 42 | 2 |
| | Metazoa | 179944 | 1491012 | 30758 57.89% | 22373 42.11% | 1329065 89.14% | 25535 1.71% | 136412 9.15% | 0.5 | 321 | 129 |
| Phylum | Apicomplexa | 239 | 1863 | 1 0.39% | 255 99.61% | 1 0.05% | 0 0% | 1862 99.95% | 1 P | 0 | 1 |
| | Arthropoda | 120479 | 1198981 | 18912 62.93% | 11141 37.07% | 1100822 91.78% | 22478 1.87% | 76064 6.34% | 0 FL | 281 | 108 |
| | Chordata | 30761 | 91785 | 10451 99.83% | 18 0.49% | 91759 99.97% | 0 0% | 26 0.03% | 0 FL | 12 | 1 |
| | Nematoda | 3437 | 30127 | 21 0.63% | 3289 99.37% | 1746 5.79% | 1196 3.97% | 27185 90.23% | 1 P | 2 | 11 |
| | Platyhel- minthes | 4459 | 22683 | 7 0.1% | 7086 99.9% | 175 0.77% | 0 0% | 22508 99.23% | 0 P | 0 | 5 |
| Class | Insecta | 91256 | 989572 | 17841 62.44% | 10734 37.56% | 1022747 94.63% | 976 0.09% | 57105 5.28% | 0 FL | 245 | 77 |

Table 4.4: Some selected phyla (subtrees), only leaf nodes, are examined for the evolution of the amount and percentage of given data to predicted data.

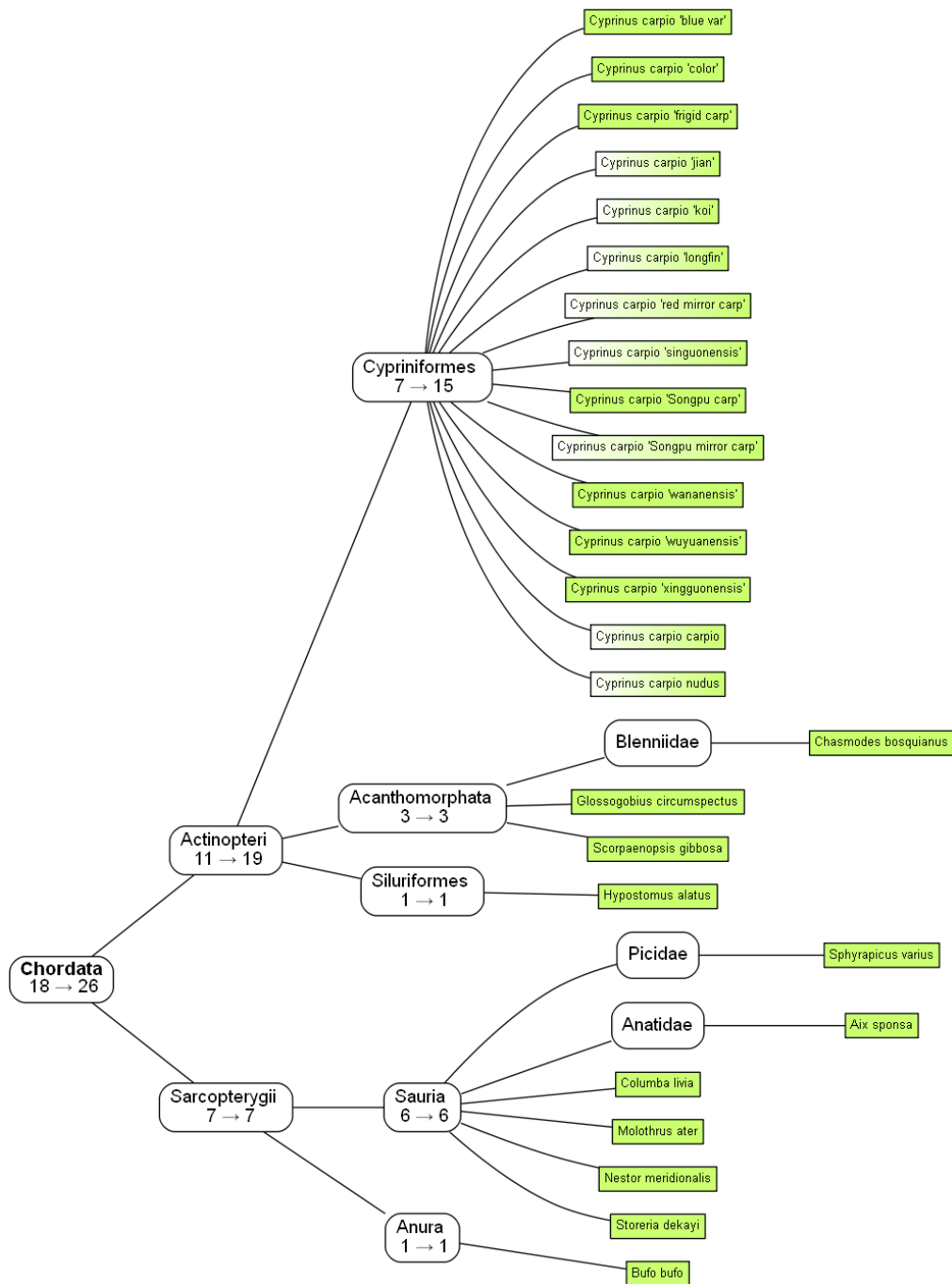


Figure 4.3: Parasites of Phylum: Chordata.

All parasite data of the chordata are mapped into a rough taxonomy (phylum, class, order, family) in order to understand their distribution and affiliation.

The internal nodes are the wanted taxa from OTL (with the addition of # input parasites to → # predicted parasites).

The leaf nodes are the input parasites (green) and the predicted parasites (white → green).

are breeding parasites, like the cuckoo and clepto-parasites as the skuas [18]. We got 6 input parasites from GloBI and there are no predictions: A woodpecker - *Sphyrapicus varius* and a duck - *Aix sponsa*, a cow bird - *Molothrus ater* known as broodparasite and some others. An example of the amplification of mistakes here are the carp. There is a paper from which GloBI concludes: Grass carp (*Ctenopharyngodon idella*) has Pathogen common carp (*Cyprinus carpio*)¹. Since there is hardly any information about free-living, it follows that all siblings are also predicted to be parasitic.

The Apicomplexa are a parasitic phylum. We found only one input organism: *Stemonitis fusca* as a free-living species. In GloBI it is listed as being parasitized by *Nectria candelaris* and *Nectriopsis sporangiicola*². The algorithm has not predicted new free-livings.

Most species of Platyhelminthes (flatworms) are parasites, although there are also free-living, predatory feeding species. These are summarized in the Turbellaria, while the parasites are divided into three other classes [19]. This also corresponds to our observations. There is one class (Rhabditophora) that contains all but one single exception of free-living species of this phylum, which includes the Turbellaria.

It should be noted, however, that this classification is outdated, as it has been proven that the Turbellaria are not monophyletic. But we will not go into that here.

For the Platyhelminthes we have more state information for the leaf nodes compared to the other considered subtrees, see Table 4.1. We start with 0.1% free-livings and predicted 0.77% as free-living species.

With the Nematoda it looks more complicated. Large parts of Nematoda are free-living, but we found only 5.32% of them. Blaxter et al. estimates the order of 25,000 parasites in the Nematoda [20] and speaks of at least seven independently arose parasitism [21]. In a recent article Blaxter identifies 18 origins [20] in Nematoda.

The problem at this point, however, is: Hallan speaks of the fact that only 23,000 species are described by the Nematoda but it is assumed that one million or more species are in this phylum.³ **TODO: Link ist nicht erreichbar!** The parasites have been much more

¹<https://www.globalbioticinteractions.org/?interactionType=hasParasite&targetTaxon=Cyprinus%20carpio>

²<https://www.globalbioticinteractions.org/?interactionType=parasiteOf&targetTaxon=Stemonitis%20fusca>

³J. Hallan, unpublished; <https://insects.tamu.edu/research/collection/hallan/>

studied and thus we start with only 0.63% (table 4.4) free-living species. Against such a shifted data situation, the algorithm is almost powerless to make correct predictions. And yet the percentage has increased to 5.34%.

TODO: Im Folgenden folgen 3 weitere ähnliche tabellen. Einmal eine ähnliche Tabelle inklusive interner Knoten 7.3, zweimal die Übersicht über die Kingdoms Blattknoten ?? bzw interne Knoten 7.4. Welche nehmen wir? Rest Appendix oder ganz raus?

Origins and Losses

Weinstein and Kuris have been searching for origins of parasitism in Animalia [22]. They identified 223 parasitic origins: 223 in Metazoa \supset 143 in Arthropoda \supset 87 in Insecta.

This has led us to count the origins and losses of parasitism in our investigation as well.

We count only one origin / loss in a parent node with different children's nodes.

Here we have encountered a problem: The Castor algorithm gives us probabilities for states.

That means there are also nodes with state like 0.3 or 0.5. The question is, how to count these state changes. We decided to round the values.

In Table ?? we can see, that we found some more origins than Weinstein and on top of that some losses.

When more closely regarding the same phyla as in the section before: Chordata, Nematoda, Platyhelminthes and Apicomplexa.

Chordata are full of free-living species and so we see only a few origins of parasitism. The root and mostly all species are predicted as free-living.

In Apicomplexa and the Platyhelminthes also look credible. Our algorithm gives us only one loss of parasitism in Apicomplexa and five in the Platyhelminthes. They are both from the root over mostly all species predicted as parasites.

Nematoda is again full of problems. The root node is predicted as a parasite and so we have more losses of parasitism for the less information of free-living species in this phylum. The rest is parasitic

As we have already mentioned Blaxter et al. found at least seven origins of parasitism [21]. If we assume that the root node of Nematoda is free-living, then some losses would have to turn around and become Origins. So it could be that we end up in a similar size as Blaxter.

4.3.2 Leave-100-out cross-validation

For a further validation of our results, we carried out a leave-100-out cross-validation. In order to achieve about 15% of the 60,871 input node states with a validation, we randomly left out 100 states 100 times. To make smaller amounts up to leave-one-out had too much computational effort. **TODO: warum 15%?**

Of these 10,000 nodes, 9,238 are unique. From the unique nodes, we predicted approximately 98.17% correct and thus 1.82% wrong, with duplicate draws always having the same prediction (data not shown).

We have again dealt with the question of how this data is best modeled. The influence of the taxa (kingdom, phylum, class) and the depth of the leaf nodes is re-modeled and the BICs compared (table 4.5). Lower taxa than classes (e.g. order) are computationally too expensive to calculate.

TODO: table for these numbers?

| Model / Taxa | Kingdom | Phylum | Class |
|------------------------------------|---------|--------|--------|
| correctly predicted ~ taxa | 117936 | 112242 | 111733 |
| correctly predicted ~ taxa + depth | 117776 | 111304 | 111273 |
| correctly predicted ~ taxa * depth | 117709 | 111262 | 113135 |

Table 4.5: BIC (Bayesian information criterion) values of cross-validation prediction models. These models are created with the R function *glm()* and compared with the *BIC()* function. This results in the listed BIC values, presented in a heatmap colorization.

The BIC values this time did not prove that the finest model is the best. Of our calculated models, *correctly predicted* \sim *phylum* * *depth* has the smallest value.

We examined the influence of the omitted data on the prediction. On average, about twice as many leaf nodes are predicted differently. The variance is very high. This means that we have a high degree of dispersion and thus a stochastic situation exists. It also describes the width of the present probability function. The standard deviation is about five times as high as the number of omitted nodes, so the variability is quintupled. Table 4.6 shows these results.

| | | min | max | mean | variance (σ^2) | σ |
|-----------------|-----------------------|-----|---------|--------|-------------------------|----------|
| distance | all | 0 | 3587.70 | 224.96 | 313650.61 | 560.05 |
| | leaf nodes | 0 | 3021.12 | 208.69 | 248103.38 | 498.10 |
| | internal nodes | 0 | 566.58 | 16.28 | 4927.95 | 70.20 |
| lost | all states | 100 | 100 | 100 | 0 | 0 |
| | FL states | 44 | 66 | 57.25 | 19.50 | 4.42 |
| | P states | 34 | 56 | 42.75 | 19.50 | 4.42 |

Table 4.6: Statistics about the leave-100-out cross-validation

The distance between original and new states is calculated using the Euclidean metric. This is summed over all states / all leaf node states and all internal node states. The lower half of the table describes the distribution of the 'lost' states between parasites (P) and free-livings (FL).

5 Discussion

TODO: Aus den models kann man auch schließen: highly variable throughout our tree

TODO: noch irgendwo einfügen:

The number of nodes in the tree corresponding to a taxonomic rank is tabulated. For the classical taxonomic ranks phylum, class, order, family, genus xyz is found (appendix 5.2).

This chapter deals with the question of how trustworthy our result of the ancestral state reconstruction of the Eukaryota tree is. And further on how well our simulation can simulate the real problem and thus make statements about the predictive power of the applied Sankoff algorithm.

We have pursued this question in various investigations and yet, of course, further possibilities for improvement remain. Despite these possibilities for improvement, our reconstruction gives a first good assessment of the whole tree.

TODO: Why is the study important?

TODO: How does this study relate to previous studies?

We evaluated our ancestral state reconstruction and the relational state prediction using three methods: a simulation, a selective analysis from a biological perspective and a leave-100-out cross-validation.

Each theme has its own limitations.

There are various expansion options and points of criticism for the simulation, which in particular revolve around the question of how well we can simulate our real data. We dedicate this question to the subsection 5.2.

Above all, the 'biological view' gives a first impression of the credibility of the results. But he can not make any statistical statements. Here the interested researcher may have to analyze the subtrees of relevant to him for himself.

Of course, cross-validation can be expanded. With more computing power, a leave-1-out cross validation could also be performed. However, our result on this point yields a strong result with 98.17% correct prediction that this does not seem necessary.

One last limitation also exists in the data situation itself. We have pointed out in various sections that we have a strong multifurcation and only a few data about leaf node states. In figure 4.2 you can see how the predictive power can improve if we have more data available. We discuss this point in section 5.1.

5.1 Data situation

The used eukaryotic synthesis tree from OTL [1] has 241,974 internal nodes and 293,463 leaf nodes. In addition, we could specify 25,962 parasitic and 34,860 free-living species from GloBI [15].

This gives us a high number of missing internal nodes (high multifurcation) and a low number of node states of the leaf nodes. In table 4.1 we have written down this percentage including some subtrees.

The investigation of the taxonomy revealed that the OTL tree has three kingdoms: Chloroplastida, Metazoa, Fungi, 53 phyla, 195 classes and 924 orders.

Since the analysis of the tree is not part of this work, it should be mentioned here that, according to recent findings, this is not complete and we lack some taxa in every rank. For example, Cavalier-Smith says that one distinguishes between seven and nine kingdoms [23]. In [section/page 39](#) of the appendix is a list of all phyla.

Also, the global database is not infallible. We found out (section [x](#)) that there is some misinformation.

We are able to report some of these directly to Globi.

We found also 57,352 (not distinct!) source species and 809,993 (not distinct!) target species without OTT indentifiers. Since we currently use only OTT indentifiers, we could not use this information.

At this point there is thus the possibility to use more of the existing data, if one performs a matching with the other indentifiers.

5.2 Simulation

TODO: einbauen.... As mentioned above, in an ancestral state reconstruction, transitions are minimized, with transition probabilities playing a role.

This subsection deals with these transition probabilities from free-living (hereinafter / as a formula FL) to parasitic (hereinafter P) and vice versa: $\mathcal{P}(FL \rightarrow P)$, $\mathcal{P}(P \rightarrow FL)$.

Different parasite types have different transition probabilities. It is very difficult to make a statement about these probabilities. It is generally assumed in this work that there are 40% parasites and 60% free-livings which is based on the estimates by Windsor [24] and $\mathcal{P}(FL \rightarrow P) = \mathcal{P}(P \rightarrow FL)$, as a result of the diversity of parasites and the lack of general determinations for this. These parameters are debated in subsection 5.2.1 of the discussion.

However, the used castor package [8] offers the possibility to enter different transition probabilities.

Wieviele Übergänge gab es dabei? Hast du die gleich (ähnlich groß) gehalten? Der Anteil von Parasiten und Freilebenden ist ja nicht gleich der Transition probability. Das sind zwei verschiedene Dinge...auch wenn die Transition probability den Anteil ergibt. neben dem Anteil der P/FL ist auch die Anzahl der Übergänge zu berücksichtigen! 50/50 kann man in einem Baum mit 1000 nodes mit einem einzigen Übergang erreichen, der die eine Hälfte des Baumes ändert. 50/50 kann man aber genauso gut mit einem Übergang auf JEDER edge erreichen. Dann würde sich immer ein P mit einem FL knoten abwechseln....

... für die Diskussion!

TODO: —

The aim of the simulation is to test the influence of various unknown or unsafe parameters in order to test the credibility of the prediction.

Some of these influences could not be tested: Firstly, the distribution of parasites and free-livings in the tree produces various parameters that we could not test all (see subsection 5.2.1) and, secondly, we discuss the simulation of a phylogenetic tree on which the evaluation based on certain parts (see subsection 5.2.2).

5.2.1 Transition probabilities

We assume that different parasite types have different transition probabilities. Establishing a common distribution across the eukaryotic tree is therefore difficult.

Based on the estimates of Windsor [24], we have assumed a distribution of 40% parasites to 60% free livings in this work. As a result of the diversity of parasites and the lack of generalizations, we have generally stated that $\mathcal{P}(FL \rightarrow P) = \mathcal{P}(P \rightarrow FL)$. But it is also reasonable to assume that in general $\mathcal{P}(FL \rightarrow P) > \mathcal{P}(P \rightarrow FL)$, because a reverse mutation is usually less likely. However, one would have to determine how much this difference is and thus discuss another parameter.

In the simulation, we tested different beta distributions with different thresholds and found that this combination has a considerable influence on the predictive power of the algorithms. At this point it would be possible to test other distributions with equal threshold values or different threshold values with equal distributions.

However, if you choose a strong free-living distribution and balance it with the threshold to achieve a 40:60 parasite free-living distribution, for example, this could be very computationally expensive.

With these further simulations you could find out if the issue of distributions plays a big role. Conversely, one could estimate possible distributions based on the data location in the tree. However, this is likely to be very difficult given the poor data. For this reason, we have decided in this work to accept very general values and not to speculate much.

5.2.2 Simulation of a phylogeny

In our simulation, we start with the simulation of a phylogeny and then depict our data situation.

For this first step we use the *randomized()* function of the phylo package from biopython [17]. The question is how close is such a randomized tree to a phylogeny.

The problems that can arise are that different species develop at different rates. This means that there could be sections in the tree with many branches (for example, subtrees of unicellular species), ie with high depths and opposite sections (of very complex species, for example). It can thus be assumed that the tree is not balanced.

For more precise statements, one would have to take deeper undercurrents, which was not possible in the context of this work.

TODO: It should be noted that we have optimized the distributions to the proportions and thus the number of state changes will be relatively high. With a smaller number of state changes, the predictive power of both algorithms would be even better.

5.3 Conclusion

From the simulation, we can conclude that we predict correctly about 60% of the nodes in the present data situation (see section 4.2). The leave-100-out cross-validation even showed that we predict the omitted nodes to be 98.17% percent correct (see section 4.3.2).

This allows for the assumption that the data is grouped and not uniformly distributed and thus smaller subtrees are present in which data are to be found in the simulation with smaller multifurcation and smaller value for unknown nodes. This is also confirmed by our biological analysis of the Chordata subtree (see subsection 4.3.1).

However, this means that the ancestral states' data in the direction of root node are probably particularly unbelievable. Which makes the localization of Origins direction root node

difficult. The question remains, how much this affects the estimation of the number of origins. Our comparison with the paper from Weinstein and Kuris [22] (see subsection 4.3.1), however, leaves us with optimism.

6 Bibliography

- [1] Hinchliff, C. E. et al. Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proceedings of the National Academy of Sciences* **2015**, *112*, 12764–12769.
- [2] Camin, J. H.; Sokal, R. R. A Method for Deducing Branching Sequences in Phylogeny. *Evolution* **1965**, *19*, 311–326.
- [3] Royer-Carenzi, M.; Pontarotti, P.; Didier, G. Choosing the best ancestral character state reconstruction method. *Mathematical Biosciences* **2013**, *242*, 95 – 109.
- [4] Felsenstein, J. *Inferring Phylogenies*; Sinauer, 2003.
- [5] Farris, J. S. Methods for Computing Wagner Trees. *Systematic Biology* **1970**, *19*, 83–92.
- [6] Fitch, W. M. Toward Defining the Course of Evolution: Minimum Change for a Specific Tree Topology. *Systematic Biology* **1971**, *20*, 406–416.
- [7] Sankoff, D. Minimal Mutation Trees of Sequences. **1975**, *28*.
- [8] Louca, S.; Doebeli, M. Efficient comparative phylogenetics on large trees. *Bioinformatics* **2017**, btx701.
- [9] Goberna, M.; Verdú, M. Predicting microbial traits with phylogenies. *The Isme Journal* **2015**, *10*, 959 EP –, Original Article.
- [10] Cunningham, C. W.; Omland, K. E.; Oakley, T. H. Reconstructing ancestral character states: a critical reappraisal. *Trends in Ecology & Evolution* **1998**, *13*, 361 – 366.
- [11] Diego Vázquez, R. N., Jeremy Goldberg Interaction Web Database (IWDB). 2003; <https://www.nceas.ucsb.edu/interactionweb/>.

- [12] Webs on the Web (WOW): 3D visualization of ecological networks on the WWW for collaborative research and education. 2004; pp 5295 – 5295 – 9.
- [13] Myers, P.; Espinosa, R.; Parr, C. S.; Jones, T.; Hammond, G. S.; Dewey, T. A. The Animal Diversity Web (online). 2018; <https://animaldiversity.org>.
- [14] Cohen, J. E. c. Ecologists' Co-Operative Web Bank. Version 1.1. Machine-readable database of food webs. *New York: The Rockefeller University* **2010**,
- [15] Poelen, J. H.; Simons, J. D.; Mungall, C. J. Global biotic interactions: An open infrastructure to share and analyze species-interaction datasets. *Ecological Informatics* **2014**, *24*, 148 – 159.
- [16] Xiang, Z.; Mungall, C.; Ruttenberg, A.; He, Y. Ontobee: A linked data server and browser for ontology terms. *Neoplasia* **2011**, *833*, 279–281.
- [17] Cock, P. J. A.; Antao, T.; Chang, J. T.; Chapman, B. A.; Cox, C. J.; Dalke, A.; Friedberg, I.; Hamelryck, T.; Kauff, F.; Wilczynski, B.; de Hoon, M. J. L. Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics* **2009**, *25*, 1422–1423.
- [18] Rothschild, M.; Clay, T. *Fleas, Flukes & Cuckoos; a Study of Bird Parasites*; New York, Macmillan,, 1957; p 368, <https://www.biodiversitylibrary.org/bibliography/6413>.
- [19] Ax, P. Verwandtschaftsbeziehungen und Phylogenie der Turbellarien. *Ergebnisse der Biologie*. Berlin, Heidelberg, 1961; pp 1–68.
- [20] BLAXTER, M.; KOUTSOVOULOS, G. The evolution of parasitism in Nematoda. *Parasitology* **2015**, *142*, S26–S39.
- [21] L. Blaxter, M.; De Ley, P.; Garey, J.; Liu, L. X.; Scheldeman, P.; Vierstraete, A.; R. Vanfleteren, J.; Mackey, L.; Dorris, M.; Frisse, L.; Vida, J.; Thomas, W. A molecular evolutionary framework for the phylum Nematoda. **1998**, *392*, 71–5.
- [22] Weinstein, S. B.; Kuris, A. M. Independent origins of parasitism in Animalia. *Biology Letters* **2016**, *12*.
- [23] Cavalier-Smith, T. Eukaryote kingdoms: Seven or nine? *Biosystems* **1981**, *14*, 461 – 481.

- [24] Windsor, D. A. Controversies in parasitology, Most of the species on Earth are parasites.
International Journal for Parasitology **1998**, 28, 1939–1941.

7 Appendix

7.1 Methods overview

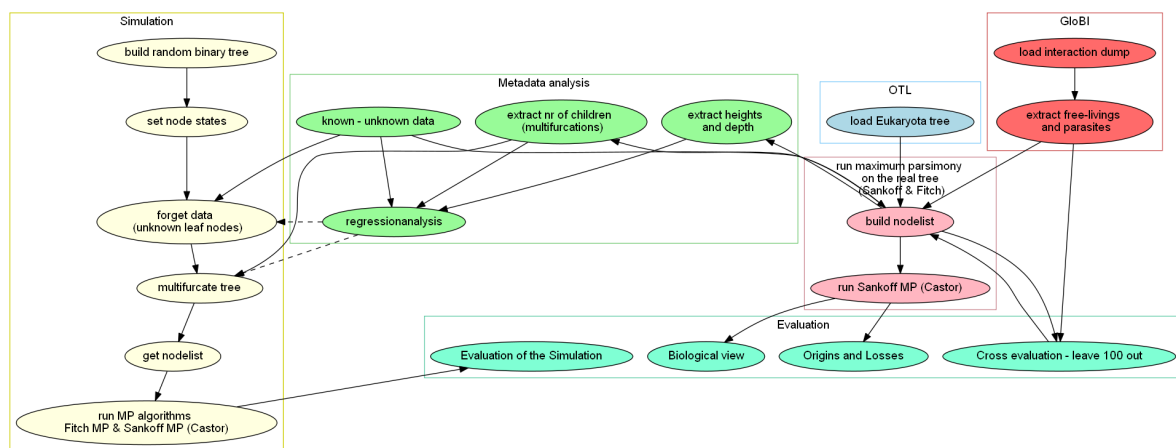


Figure 7.1: Big overview of the whole Workflow

7.2 OTL analysis

7.2.1 List of all phyla

Phyla (53):

Acanthocephala, Amoebozoa, Apicomplexa, Arthropoda, Ascomycota, Bacillariophyta, Basidiomycota, Brachiopoda, Bryozoa, Chaetognatha, Chlorophyta, Chordata, Chromerida,

Chytridiomycota, Ciliophora, Cnidaria, Colponemidia, Ctenophora, Cyclophora, Echinodermata, Entoprocta, Entorrhizomycota, Euglenida, Foraminifera, Gastrotricha, Glomeromycota, Gnathostomulida, Haplosporida, Haptophyta, Hemichordata, Kinorhyncha, Loricifera, Microsporidia, Mollusca, Myzostomida, Nematoda, Nematomorpha, Nemertea, Onychophora, Orthonectida, Phaeophyceae, Picozoa, Placozoa, Platyhelminthes, Porifera, Priapulida, Rhodophyta, Rhombozoa, Rotifera, Streptophyta, Tardigrada, Xanthophyceae
Wobei von Streptophyta -> Anthocerotophyta, Marchantiophyta, Bryophyta, Tracheophyta als Phylum im Phylum gefunden und nicht einbezogen wurden und Magnoliophyta als Phylum in Tracheophyta ebenfalls nicht.

Distribution of Taxa

- In the tree we can distinguish 28 different Taxa with the OTL taxonomic tree.
- The most of them are hardly represented. The major taxonomic groups are: ...
- Here **you** can see some characteristics of the Multifurcation of the tree.

In a phylogeny, the taxonomic division of the tree is far too coarse, meaning that there

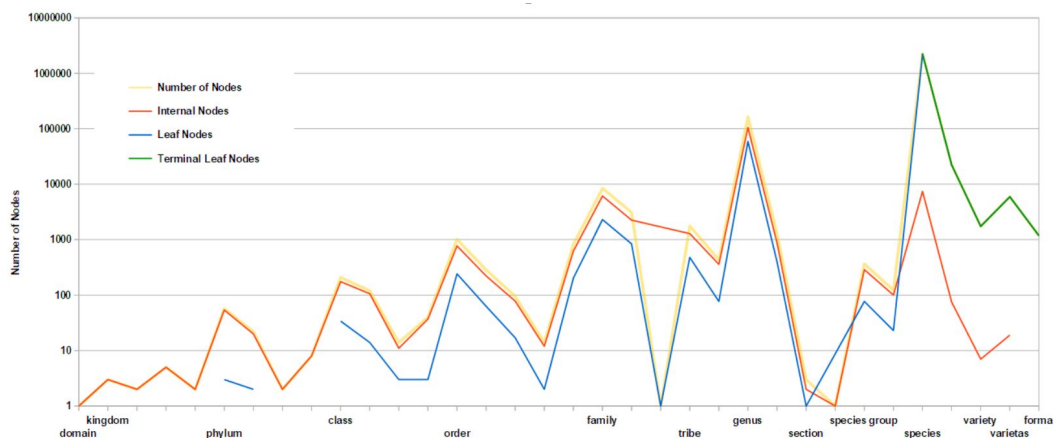


Figure 7.2: Distribution of Nodes in Rank-Categories

should be more subtaxa or 'unranked' nodes. But the closer we get to the root, the more the pure taxonomic tree is reflected. If the tree are binary, the taxa would have to double. But the multipliers for some are much bigger and for others much smaller, which **you** can

see in in figure 7.2.

... (see Table 7.2)

extended leaf nodes (real leaf nodes)

Distribution of data in the taxa

Mithilfe des taxonomischen Baums von OTL haben wir die Knoten ihren Kingdoms, Phyla und Classes zugeteilt (see Table 7.2).

7.3 Results

| Taxa | Number of Nodes | Internal Nodes | Leaf Nodes | Terminal Leaf Nodes |
|--------------------|-----------------|----------------|------------|---------------------|
| domain | 1 | 1 | | |
| kingdom | 3 | 3 | | |
| subkingdom | 2 | 2 | | |
| infrakingdom | 5 | 5 | | |
| superphylum | 2 | 2 | | |
| phylum | 57 | 54 | 3 | |
| subphylum | 22 | 20 | 2 | |
| infraphylum | 2 | 2 | | |
| superclass | 8 | 8 | | |
| class | 209 | 175 | 34 | |
| subclass | 120 | 106 | 14 | |
| infraclass | 14 | 11 | 3 | |
| superorder | 40 | 37 | 3 | |
| order | 1014 | 772 | 242 | |
| suborder | 285 | 222 | 63 | |
| infraorder | 95 | 78 | 17 | |
| parvorder | 14 | 12 | 2 | |
| superfamily | 829 | 626 | 203 | |
| family | 8449 | 6143 | 2306 | |
| subfamily | 3090 | 2250 | 840 | |
| supertribe | 1 | 0 | 1 | |
| tribe | 1764 | 1285 | 479 | |
| subtribe | 435 | 359 | 77 | |
| genus | 164656 | 105452 | 59204 | |
| subgenus | 1266 | 869 | 397 | |
| section | 3 | 2 | 1 | |
| subsection | 1 | 1 | 0 | |
| species group | 365 | 288 | 77 | |
| species subgroup | 123 | 100 | 23 | |
| species | 2247251 | 7423 | 2239828 | 2228993 |
| subspecies | 22437 | 75 | 22362 | 22239 |
| variety | 1755 | 7 | 1748 | 1726 |
| varietas | 5970 | 19 | 5951 | 5909 |
| forma | 1181 | | 1181 | 1181 |
| no rank | 954 | 719 | 235 | 7 |
| no rank - terminal | 37452 | | 37452 | 37452 |
| (no entry) | 40099 | 40099 | | |

Table 7.1: TODO: ...

| Kingdom (3) | Number of Nodes | Phylum (25) | Number of Nodes | max max height |
|----------------|-----------------|-----------------|-----------------|----------------|
| Metazoa | 1 465 207 | Arthropoda | 1 170 539 | 54 |
| | | Chordata | 106 650 | 74 |
| | | Mollusca | 80 022 | 22 |
| | | Platyhelminthes | 27 141 | 9 |
| | | Nematoda | 24 564 | 23 |
| | | Cnidaria | 14 878 | 36 |
| | | Porifera | 11 737 | 26 |
| | | Echinodermata | 10 654 | 14 |
| | | Bryozoa | 8 631 | 11 |
| | | Rotifera | 3 093 | 7 |
| | | Nemertea | 1 793 | 7 |
| | | Tardigrada | 1 654 | 7 |
| | | Acanthocephala | 1 596 | 6 |
| | | Brachiopoda | 1 055 | 9 |
| | | Nematomorpha | 633 | 7 |
| | | Chaetognatha | 360 | 7 |
| | | Hemichordata | 196 | 5 |
| | | Cycliophora | 11 | 5 |
| Fungi | 254 871 | Ascomycota | 157 045 | 19 |
| | | Basidiomycota | 92 931 | 18 |
| | | Microsporidia | 1 949 | 6 |
| | | Glomeromycota | 1 490 | 6 |
| | | Chytridiomycota | 1 456 | 6 |
| | | | | |
| Chloroplastida | 121 239 | Streptophyta | 120 731 | 49 |
| | | Chlorophyta | 508 | 6 |

Table 7.2: TODO: ...

| Phylum | # nodes | original states | | final states | | | | | |
|-----------------|---------|-----------------|------|--------------|-----|------|------|------|-------|
| | | FL | P | 0 (FL) | 0.4 | 0.5 | 0.67 | 0.75 | 1 (P) |
| Chordata | 122546 | 10451 | 18 | 122473 | 0 | 0 | 0 | 0 | 73 |
| Nematoda | 33564 | 21 | 3289 | 846 | 0 | 1133 | 0 | 0 | 31585 |
| Platyhelminthes | 27142 | 7 | 7086 | 1010 | 0 | 175 | 0 | 0 | 25957 |
| Apicomplexa | 2102 | 1 | 255 | 1 | 0 | 0 | 0 | 0 | 2101 |

Table 7.3: Some selected phyla (subtrees), including internal nodes, are examined for the evolution of the amount and percentage of given data to predicted data.

| Kingdom | # nodes | original states | | final states | | | | | | | |
|----------------|---------|-----------------|-------|--------------|------|-------|------|-------|------|------|--------|
| | | FL | P | 0 (FL) | 0.25 | 0.33 | 0.4 | 0.5 | 0.67 | 0.75 | 1 (P) |
| none | 84456 | 45 | 529 | 15035 | 243 | 25910 | 0 | 8764 | 6183 | 0 | 28140 |
| Fungi | 324105 | 577 | 2983 | 39088 | 0 | 0 | 0 | 5858 | 0 | 0 | 274803 |
| Chloroplastida | 460457 | 3519 | 77 | 454211 | 0 | 0 | 0 | 4688 | 0 | 0 | 1558 |
| Metazoa | 1670956 | 30758 | 22373 | 1485749 | 0 | 0 | 1313 | 29002 | 5102 | 1957 | 147833 |

Table 7.4: All kingdoms (subtrees), including internal nodes, are examined for the evolution of the amount and percentage of given data to predicted data.