

Freie Universität Berlin

Fachbereich Mathematik und Informatik

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

using a large multifurcated phylogenetic synthesis tree

Eingereicht am:
3.4.2018

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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 some estimates, above 50% of all eukaryotes are parasites. Nevertheless, it is difficult to obtain information whether a particular taxon is a parasite computationally 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 25,992 and 34,879 species as parasites and free-living, respectively and predict states for over 2.7 million (97.6%) leaf nodes without state information.

We estimate the accuracy of our maximum parsimony based predictions using cross-evaluation and simulation at roughly 80% overall, but strongly varying between clades. 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.

This study focuses on the ancestral state reconstruction of parasitism in the tree of life of Eukaryota. We predict unknown states of species and estimate origins and losses of parasitism.

The challenge here is the size of the tree and the little information about it.

Such a large phylogenetic tree does not completely exist and therefore we work with a synthesis tree of OTL [1] which is highly multifurcated.

For the 2,535,437 leaf nodes we could not gather much data. From the GloBI database [2] which we used, we could only collect 25,992 parasitic and 34,879 free-living species. It follows that we have only $\approx 2.4\%$ state information.

So far, especially small scale studies have been carried out or highly manual. In this scale, it requires different data sources to be interconnected.

We performed an analysis of existing algorithms and selected a Sankoff maximum parsimony algorithm using the R package *Castor* [3].

Nevertheless, the results are convincing and even though purely computational approach which did not include human experts input, results coincide with prior knowledge. Also regarding the number of events, our estimates coincide with previous results by human experts, e.g. the study by Weinstein and Kuris [4].

Anmerkung: Klassisch packt man keine Referenzen in Abstracts (bzw. wenn das meist als Kurzreferenz also (Author et al., 2017). (Bernhard)

We have compared the results of some subtrees with known knowledge (Chordata, Nematoda, Platyhelminthes and Apicomplexa) and, except for the Nematoda, the results looked very good. In the case of Nematoda, the data situation is strongly shifted to the few parasites.

We could partly compare our number of origins with the results of Sara B. Weinstein and Armand M. Kuris from their article *Independent origins of parasitism in Animalia* and have

come to a similar magnitude. They identified 223 parasitic origins in Metazoa and we were able to estimate about 300 origins.

Contents

1	Introduction	1
2	Methods	4
2.1	Description of data sets	5
2.1.1	OTL	5
2.1.2	GloBI	6
2.2	Metadata analysis	7
2.2.1	Transition probabilities	7
2.2.2	Missing information	8
2.3	Simulation	9
2.3.1	random binary tree	10
2.3.2	simulating states and transitions between them	11
2.3.3	simulating loss of information of the tree topology	11
2.4	Ancestral state reconstruction methods	12
2.4.1	Fitch maximum parsimony	13
2.5	Implementation	16
3	Results	17
3.1	Metadata analysis - Missing information	17
3.1.1	Data artifacts	19
3.1.2	Taxa	19
3.1.3	Poisson regression of the multifurcation	19
3.1.4	Binomial regression of the unknown state information	21
3.2	Results of simulation / Influence of different parameters	22
3.3	Results of the real data analysis created with Sankoff	24
3.3.1	Biological view	24

3.3.2	Origins and Losses	28
3.3.3	Cross evaluation - leave 100 out	29
3.4	Effects of Taxa in the different models	30
4	Discussion	34
4.1	Simulation	34
	Bibliography	36
5	Appendices	39
5.1	Methods overview	39
5.2	OTL analysis	39
5.2.1	List of all phyla	39
5.3	Missing leaf state modelling - Residual tables	41

1 Introduction

This paper 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], with the application of prediction of parasitism.

We will test the existing maximum parsimony algorithms Fitch [5] and Sankoff [6] for this task and estimate their predictive power. The present tree structure of OTL is not binary. A tree is binary (or bifurcated), when each node has at most two children otherwise it is multifurcated [7].

Maximum parsimony methods are developed for phylogenies, which are usually depicted as binary trees. Parsimonious in phylogeny refers to favoring the tree that needs the least evolutionary change to explain the observed data. In our case, it is about the change of states 'is free-living' or 'is parasitic'.

The Sankoff method is implemented by Louca et al. for the non-binary case and is available as an R package called *Castor* [3]. In addition, we have implemented the Fitch method and adapted it for multifurcated trees.

This achieves that we can predict ancestral states and unknown states of living species for large non-binary relatives trees.

For about 50 years, people have been working on ancestral state reconstruction. The first paper is by Camin and Sokal, who in 1965 were working on algorithms for discrete-state data [8]. 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 multifurcated tree with **binary/two discrete** states.

Anmerkung: hier mehr dazu? Likelihood vs parsimony... das befindet sich momentan im

Methoden Teil: Ancestral state reconstruction methods

For this purpose, various studied methods and their advantages and disadvantages will be compared.

These algorithms were developed especially for the binary problem, since one has considered much smaller subtrees, of which one knows all the splits.

By developing a whole of an entire tree of life, the problem arises that it is by no means binary.

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.

To accomplish this task, a large phylogenetic tree and information about the current species states is needed.

The biggest 'phylogenetic tree' is a synthesis of phylogenetic trees filled with a taxonomic trees given by Open Tree of Life (OTL) [1]. For the information about the current states of the species we use the interaction database Global Biotic Interactions (GloBI) [2]. 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.

At this point a few words to the term parasitic. There are different definitions. Since we use GloBI to classify species, we use their definition of parasitism. Again, in GloBI, Ontobee definitions are used [10]. 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." [?]. This definition includes: ecto- and endoparasites, parasitoids, kleptoparasites and pathogenes. **TODO: Mungall, C., (2017). Definition for the interaction-term: "parasitised by; has parasite" ob ontobee.org. Last checked: 24.07.2017 at ontobee.org/ontology/...**

The objectives of this work are the following points: (1) Find a suitable ancestral state reconstruction method. (2) Perform reconstructing on the Eucaryotic synthesis tree of OTL. The goal of Point 1 is to evaluate the possible methods based on a simulation of our data situation.

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

2 Methods

As initiated, in this paper, a maximum parsimony algorithm is applied to the whole tree of life to obtain an ancestral state reconstruction of free-living versus parasite states.

So far, these reconstructions have been made mainly on binary trees with better data availability. Therefore, a simulation is first performed to evaluate existing algorithms and decide how they may be adapted to our given problem. This is to perform the ancestral state reconstruction for a multifurcated (non-binary) tree using binary states.

Accordingly, in addition to the necessary data sets (GloBI, OTL), the chosen algorithm and the evaluation of its results, this chapter also deals with the previously performed simulation and the evaluation of the various algorithms and their parameters.

Figure 2.1 briefly outlines these relationships.

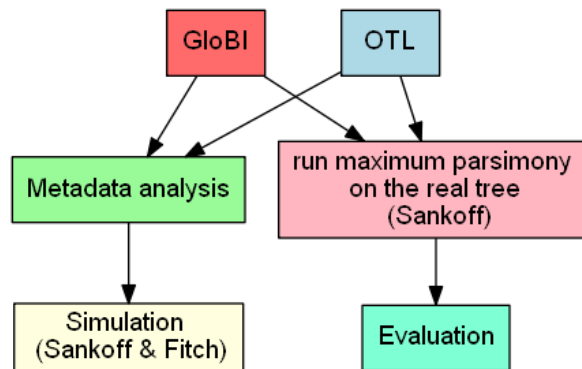


Figure 2.1: Workflow

The resulting procedure is as follows:

- (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 resulted algorithm on the original data.
- (7) Evaluate and interpret results.

TODO: In den einzelnen Abschnitten Teile von dem großen Workflow verwenden?: 5.1

2.1 Description of data sets

For **our** research two types of data are required: a tree and information about the states.

For the tree **we** decided to use Open Tree of Life (OTL) [1].

For the state information, **we** decided to use the Global biotic interaction database (GloBI) [2].

2.1.1 OTL

For **our** project a large database for phylogenetic trees and also for a taxonomic tree is needed. Since an ancestral state reconstruction algorithm is applied to the phylogenetic tree, and for the assessment and other properties the taxonomy provides much more information. OTL gives a synthesis of phylogenetic trees (currently 819 trees) and a taxonomic tree. OTL also includes the large phylogenetic database TreeBASE [1].

TODO: Das steht auf der Website nicht in dem Paper...

For phylogenetic data, there are at least five big data collections, namely:

- ITIS (Integrated Taxonomic Information System) [11]
- NCBI (National Center for Biotechnology Information) [12]
- WORMS (World Register of Marine Species) [13]
- GBIF (Global Biodiversity Information Facility) [14]
- OTT (OpenTreeOfLife-Taxonomy) [1]

TODO: In die discussion?:

ITIS is only a small set of 100 % confirmed and named species. GBIF is not composed with the help of phylogeny, the same is valid for the NCBI taxonomy. The WORMS taxonomy is a way too small dataset of mostly marine species.

Here the taxonomy from OTL is used because it is including most of the known taxonomies and is synthesised by preferring taxonomies that match with available phylogenetic data. Furthermore the team from OTL preferre a maximum number of species [1]. This is resulting in somekind of hybrid between taxonomy and phylogeny. *Anmerkung: Wie genau ist das ein Hybrid? Genauer beschreiben, was Du damit meinst... (Thilo)*

A closer look is being made to some of the features of the synthesis tree. On the one hand the distribution of the taxa and on the other the distribution of the nodes on the taxa. Since this is not directly relevant to **our** study, there is a section in the appendix 5.2.

2.1.2 GloBI

TODO: Marius: "There aren't many big active interaction databases out there, most of them are offline or outdated. For example: IWDB (Interaction Web Database) [15], Webs on the Web [16], Animal Diversity Web [17] and ecoweb [18]. GloBI is including most of the known ones and is still growing actively [2]. So the question which interaction database could be used was answered rather quickly."

This database consists of entries of the form: species A (source) interacts with B (target). A number of interactions have been identified¹, including those that the species source or target has become a parasite or a free-living species from the biological perspective. These 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
- parasite target: hasParasite, hasPathogen

¹<https://github.com/jhpoelen/eol-globi-data/.../InteractType.java>

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. The case a parasite conquers (parasitizes) another parasite yields conflicting states for the second species. This is solved by preferring parasitic.

Two lists are formed: parasites and free-livings, and the source or targets of an interaction are added to these.

2.2 Metadata analysis

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

There are two major types of parameters:

i) Biological parameters (A result of the evolutionary process.):

- transition probabilities

ii) Distribution of the loss of information:

- Loss of topology (\rightarrow mutlifurcations)
- Unknown information about states of some leaf nodes

The influence of these parameters are tested on our result using our simulation (Section 2.3).

2.2.1 Transition probabilities

This subsection deals with the 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. In general, **we** assume that there are 40 % parasites and 60 % free-livings which is based on the estimates by Windsor [19] and $\mathcal{P}(FL \rightarrow P) > \mathcal{P}(P \rightarrow FL)$, because a reverse mutation is usually less likely.

TODO: This is discussed in section x of the discussion.

For the maximum parsimony analysis of the real data, all transition probabilities are equated. However, the used castor package [3] offers the possibility to enter different transition probabilities.

In the simulation two beta distributions have been chosen and a threshold that indicates the change between states.

Different thresholds with different beta distributions are simulated, with different distributions of parasites and free-livings:

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

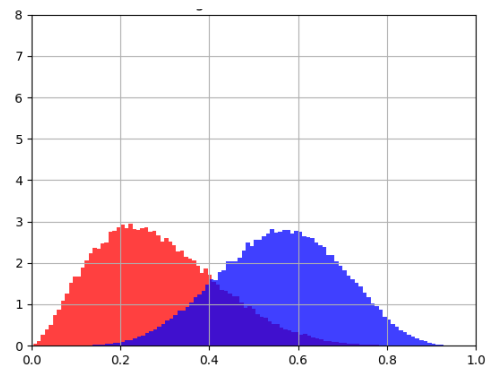


Figure 2.2: 60 % Free-living - 40 % Parasites
red: parasites, blue: free-living,
the threshold is at 0.4

(TODO: see results simulation, ref...).

Figure 2.2 shows one example of these.

TODO: Plot neu erstellen: Achsenbeschriftung, threshold

2.2.2 Missing information

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 41,974 internal nodes, that is:

$$100 - \frac{100}{(2293463 - 1) \times 41974} \approx 98.16\%$$

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.

For the present Eukaryota tree with 2,293,463 leaf nodes, 34,869 free-livings and 25,962 parasites are found, which are

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

unknown states of leaf nodes.

In the simulation, the influence of the multifurcation and missing data in leaf nodes on the predictive accuracy of the ancestral state reconstruction algorithms is tested.

For the real data, generalized linear models are compared with poisson respectively binomial regression according to their residuals and BICs.

Anmerkung: Erklären, warum und wozu das gemacht wird. Warum BIC und nicht AIC? Erklären, was beide bedeuten, wäre sinnvoll. (Thilo)

For each node, depth, min, max and mean height were noted. Where the depth of a node is the distance (number of edges) to the root node and the height of a knot is described as the largest distance to a leaf node. In this work, a distinction is made between minimum, maximum and average distance (→ min, max and mean height).

The influence in the modeling of these parameters was tested, additive as well as multiplicative.

2.3 Simulation

There are various possibilities of ancestral state reconstruction. The simulation compares different 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 [3].

TODO: Figure 2.3 shows the course of the simulation. The individual steps are explained in the following subsections: ...

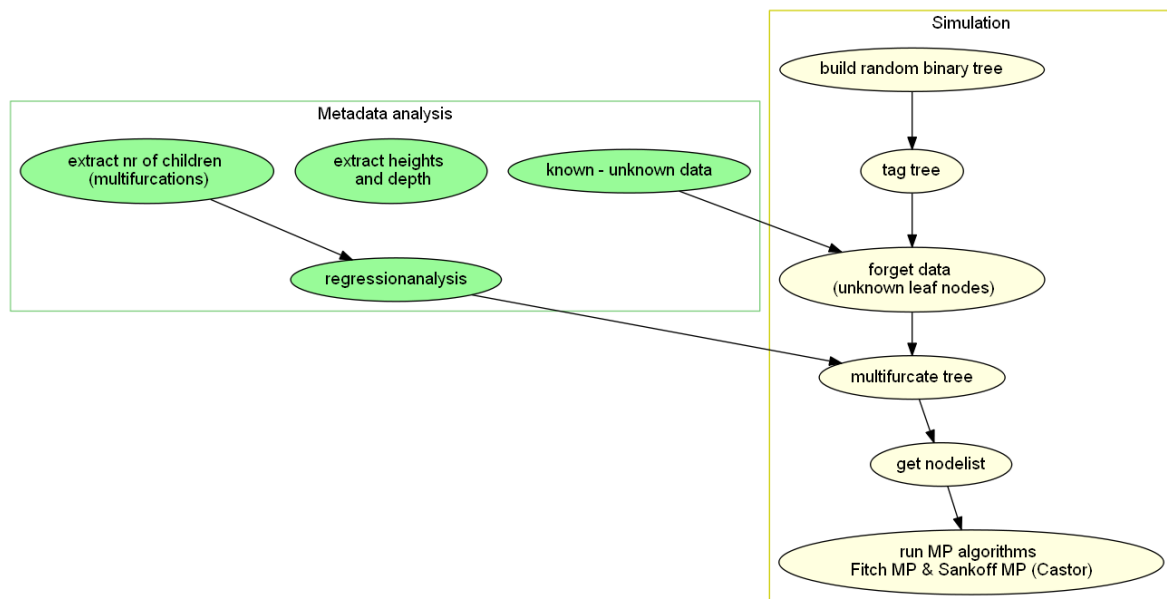


Figure 2.3: Course of the simulation with influence of the metadata analysis from the real data.

TODO: Evaluation - compare trees (distances)

2.3.1 random binary tree

A tree is needed to do a simulation of ancestral state reconstruction. It had 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 [20]. They offer a randomized function which returns a BaseTree².

TODO: ref in die discussion über die randomized function? Diskutieren wir das?

²<https://github.com/biopython/biopython/blob/master/Bio/Phylo/BaseTree.py>

2.3.2 simulating states and transitions between them

The next step is to simulate the states of the nodes using the transitions. Again, fully known states are simulated and then everything is 'forgotten' but a few in the leaf nodes so that **you** can later compare the reconstruction with the origin.

The root node is defined as ancestor of all subsequent species and in this case, determined to be free-living. Therefore, a 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 distribution until **you** get above the threshold and the new node changes state. *Anmerkung: Für den Tree/Graph Traversal würde sich eine erklärende Abbildung anbieten. Nur Text macht es sehr schwer verständlich an der Stelle. (Thilo)*

To ensure that the parameter of the binomial distribution is restricted to the $[0,1]$ interval, it is modeled with a beta distribution as in Figure 2.2.

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

Here begins the simulation of the lost information. This is on the one hand the states and on the other the topology of the tree. Some splits of nodes are unknown with which the tree is multifurcated (explained in the following section **TODO: pageref**).

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 'forgotten' and stored in another column to the node.

Different percentages of forgetting the information are simulated, as **you** can read in the **TODO: section ... from the results** .

2.3.3 simulating loss of information of the tree topology

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

2.4 Ancestral state reconstruction methods

As presented in the introduction, there are some methods for ancestral state reconstruction. For this purpose, various studied methods and their advantages and disadvantages are compared below.

Royer-Carenzi et al. distinguishes two major classes of ancestral state reconstruction methods:

The first is to explain the current state with the least number of state changes between an ancestor and his child, this is called parsimonious.

The other class she presents involves 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 [21].

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 there are no development times of species in our case, **you** can ignore this.

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. Again, this problem is irrelevant to the problem at hand, because **you** can only work with generalized models in the analysis of the entire Eukaryota tree.

Parsimony-based methods are used in this work, since they are fully sufficient for the presented use case here. Following the principle of the simpler model first.

Felsenstein [7] discusses in his book two algorithms that generalize all previous methods (from Camin and Sokal [8], **TODO: Kluge and Farris** and Farris [22]): Fitch parsimony [5] and Sankoff parsimony [6].

Anmerkung: Unter Farris war auch noch der Begriff Wagner trees in Gebrauch, als Verallgemeinerung der parsimonious trees von Camin und Sokal. (Lydia)

TODO: Wagner-parsimony [23]

Thus, the methods used in this work are those of Fitch and Sankoff. For Fitch, the algorithm has been extended from binary to multifurcated trees. For the Sankoff algorithm, Louca and Doebeli have presented an implementation for non-binary trees published in an R package named *castor* [3].

2.4.1 Fitch maximum parsimony

Fitch maximum parsimony is an algorithm for rooted, binary trees and describes an ancestral state reconstruction for discrete states [5] by minimizing transitions between states.

Note, the original Fitch algorithm has the sole purpose of minimizing the number of transitions and not reconstructing the ancestral nodes. Felsenstein [7] describes a simple extension for the reconstruction. Cunningham et al. [24] have refined these. **TODO: Wir haben mit ein paar kleinen Änderungen optimiert... und schließlich auf multifurcated angepasst... TODO: eigentlich ist Cunningham 'nur' eine kritische Neubewertung. Sie beziehen ihren Algorithmus auf Swofford und Maddison...**

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 paper, 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 (eg {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 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 / move down the tree / postorder tree traversal. Each internal node is formed from its child nodes, where at the beginning the only information lies.

Second traverse from the root node to the leafs. Each internal node is formed from its father 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 was designed to minimize transitions without predicting actual states of internal nodes, so it was 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 were all tested and compared in the simulation.

Some of these options are already available in the binary case:

- The father 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?
- Since there are several siblings, do **you** first of all make the cut or union, or directly in the whole with the father node?

The first point already has an effect on the binary case. Figure 2.4 shows both possibilities of the three sets.

Cunningham uses only the first state set of the father node [24].

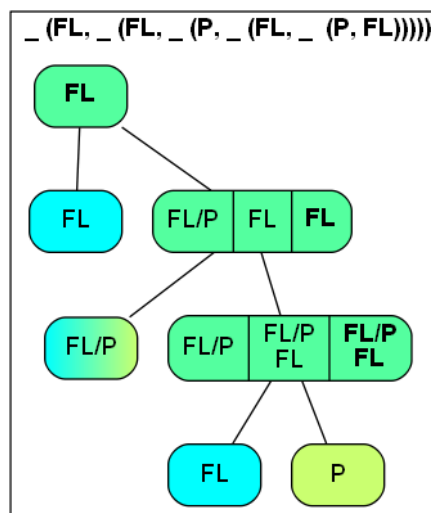


Figure 2.4: Fitch algorithm for binary trees.

The unknown leaf node is described 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.

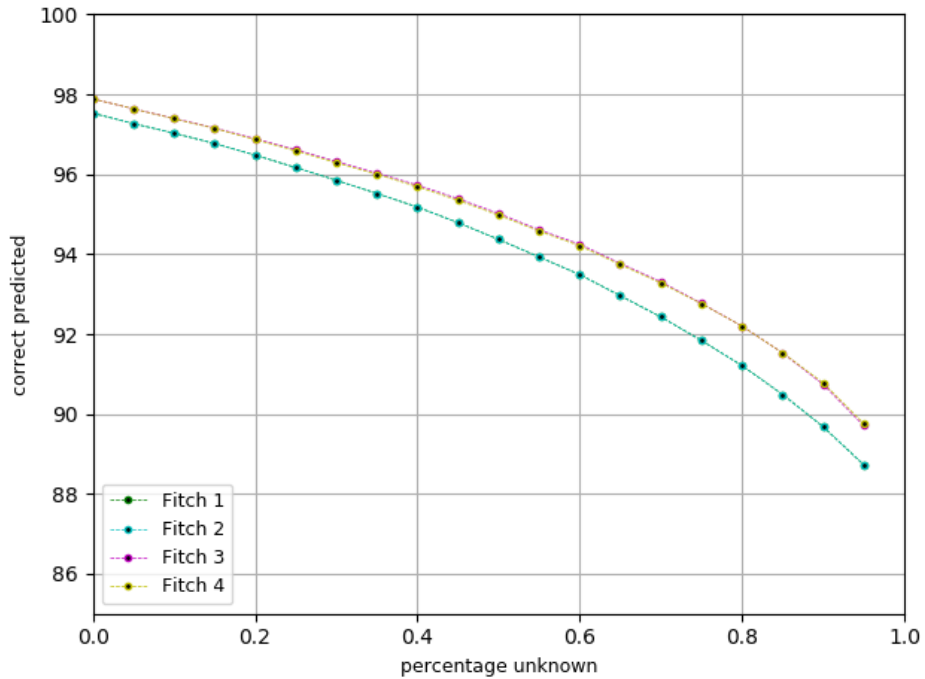


Figure 2.5: Test of Fitch Versions.

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

- i) Fitch 1: First state set of father node; intersection/union of siblings first.
- ii) Fitch 2: First state set of father node; intersection/union of siblings together with father node.
- iii) Fitch 3: Both state sets of father node; intersection/union of siblings first.
- iv) Fitch 4: Both state sets of father node; intersection/union of siblings together with father node sets.

These four versions were tested in the simulation with 100 trees and 10000 leaf nodes and a distribution of 60 % FL to 40 % P. Figure 2.5 shows this over all unknown node percentage. At 95 % unknown nodes and 95 % of multifurcation of the internal nodes, version 1 was 88.37 %, version 2 was 88.37 %, version 3 was 88.4 %, and version 4 was 88.39 % correct. Therefore, only version 3 was used for all further simulations.

Sankoff

Maximum parsimony algorithm from Sankoff implemented in the R package castor [3].

TODO: transition probabilities: all equal

2.5 Implementation

You can find the full code on GitHub: github.com/Irallia/IZW-HU-Parasites.

Most of the code was written in Python. The analyzes and the use of the Castor package in R. There are some shell scripts to execute whole workflows.

3 Results

A big point in this chapter is the result of examining the input data. How is the situation? What influence does that have on our actual result? What can we do about it? Our simulation gave us some results to this.

Otherwise, this chapter is mainly about the actual reconstruction of the states. This means, on one hand investigation of origins and losses of the inner nodes and on the other, the prediction of unknown states of leaf nodes.

3.1 Metadata analysis - Missing information

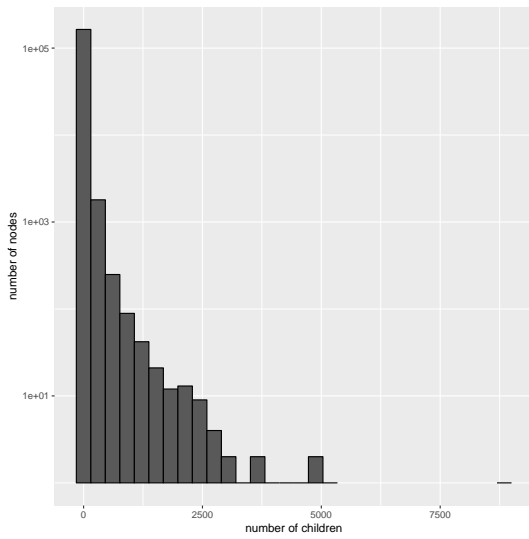
As previously presented, we have two types of missing information: unknown states of leaf nodes and multifurcation.

We examined the ridge of multifurcation of the tree. A complete phylogenetic tree would be binary, which means the number of leaf nodes is closely to the number of internal nodes. But since we only work with a synthesis tree, this tree is multifurcated: we have 241 974 internal nodes and 2 293 463 leaf nodes.

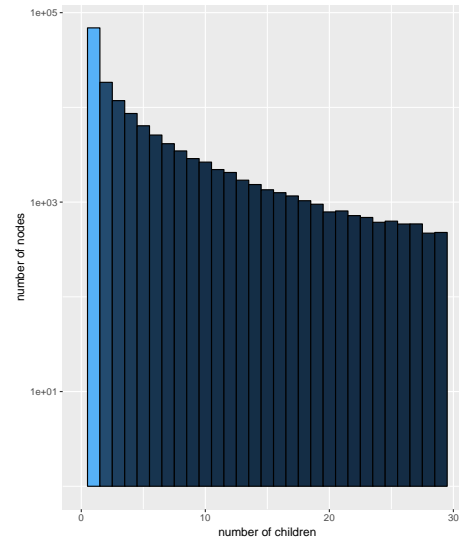
For a first overview we collected for every node its number of children (degree -1), and plotted this in two histograms, see figure 3.1.

The multifurcation affects only the internal nodes. We collected the number of children -2 of every node (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.

As **you** can see, we are very far from a binary tree.



(a) Histogram with automatic binwidth.



(b) Histogram with $\text{binwidth} = 1$.
light blue: binary;
dark blue: multifunction

Figure 3.1: Histograms about the multifurcation of the internal nodes of the synthesis tree.

Some subtrees have been examined, these have the following percentages of missing information: See table 3.1.

Subtree of	unknown states	multifurcation
Eukaryota	97.34 %	98.16 %
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 3.1: Examination of subtrees regarding missing information.
Unknown states: missing state information of leaf nodes
Multifurcation: missing internal nodes

3.1.1 Data artifacts

At this point we also found out that there are some nodes with only one child node (55,700 nodes).

These are both, the most nodes are right in front of a leaf, as well as some nodes are deep in the tree (3,956 with height > 2). They are probably a result from the fact that taxonomic information has been incorporated into a phylogeny.

Some examples:

- Nephroselmidophyceae: (class)
<https://tree.opentreeoflife.org/opentree/argus/ottol@1038762>
- Phrynocrinidae: (family)
<https://tree.opentreeoflife.org/opentree/argus/ottol@3647979>
- Elaeocarpus sylvestris:
<https://tree.opentreeoflife.org/opentree/argus/opentree9.1@ott166969>

3.1.2 Taxa

The investigation of the taxonomy revealed that our 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 [25]. In section 39 of the appendix **you** can find a list of all phyla.

3.1.3 Poisson regression of the multifurcation

First of all we measured the strength of the multifurcation with the help of the intercept: $2.821 > 0 \Rightarrow$ there is a multifunction.

Comparing the different kingdoms, we find that multifunctionality is greater in Fungi than in Chloroplastida than in Metazoa:

$$4.0999(FungiIntercept) > -0.9132(ChloroplastidaIntercept) > -1.4320(MetazoaIntercept)$$

Then we have 9 times 4 models of different complexity levels. In Table 3.2 is a deviance table,

Model / Taxa	Kingdom	Phylum	Class	Order
multifurc ~ taxa	7774454	7435700	7337241	7076068
multifurc ~ taxa + depth	7752303	7431609	7334754	7027578
multifurc ~ taxa + max.height	7730196	7375889	7275856	7005424
multifurc ~ taxa + min.height	7472500	7233486	7144686	6890703
multifurc ~ taxa + mean.height	7304402	7128318	7055313	6815271
multifurc ~ taxa * depth	7714881	7335396	7250759	6843004
multifurc ~ taxa * max.height	7692980	7311241	7187504	6795823
multifurc ~ taxa * min.height	7442387	7177002	7094933	6795099
multifurc ~ taxa * mean.height	7247309	7020258	6965794	6665565

Table 3.2: Residuals of multifurcation models

where the different residuals are listed. Since only residuals are comparable in complexity, we have also calculated the BIC values of the models in table 3.3.

The Residuals give us the error of the model. If the value is small, our data will be well

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 3.3: BIC of multifurcation models

modeled.

Within the complexity classes 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. Smaller taxa than orders (eg family) were computationally too expensive to calculate.

3.1.4 Binomial regression of the unknown state information

Next to the problem of the multifurcation of the tree is the less of data we have for the species. For the ancestral state reconstruction, we need information in the leaf nodes.

The eukaryotic synthesis tree has 293,463 leaf nodes. The GloBI database has 5 346 414 interactions (at this timepoint). Out of this data we got 51,337 distinct free-living species and 47 332 distinct parasite species → unknown nodes 2,194,794 ($\approx 95.7\%$).

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

We also compared different models in terms of their BICs (Table: 3.4). The Residuals are not very meaningful here, since all models have different complexities. For the sake of completeness, the associated deviance tables are located with the residuals in the appendix 5.3.

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

Table 3.4: BICs of unknown information

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 (eg order or family) was too expensive.

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

3.2 Results of simulation / Influence of different parameters

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

We examine different parameters. In figure 3.2 is an overview of the results.

The first column describes the distributions of free-livings and parasites with a given threshold for the respective simulations to the right.

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.

As **you** can see both algorithms are always over 50 % and therefore better than guessing. Moreover, they are usually close to each other, with Sankoff always makes better predictions except for equally distributed states as Fitch.

You can see that the distribution of states has a strong influence on the prediction. The more evenly distributed, the harder it is to predict.

If more than 60 % of internal nodes are missing, Fitch breaks significantly in his prediction compared to Sankoff.

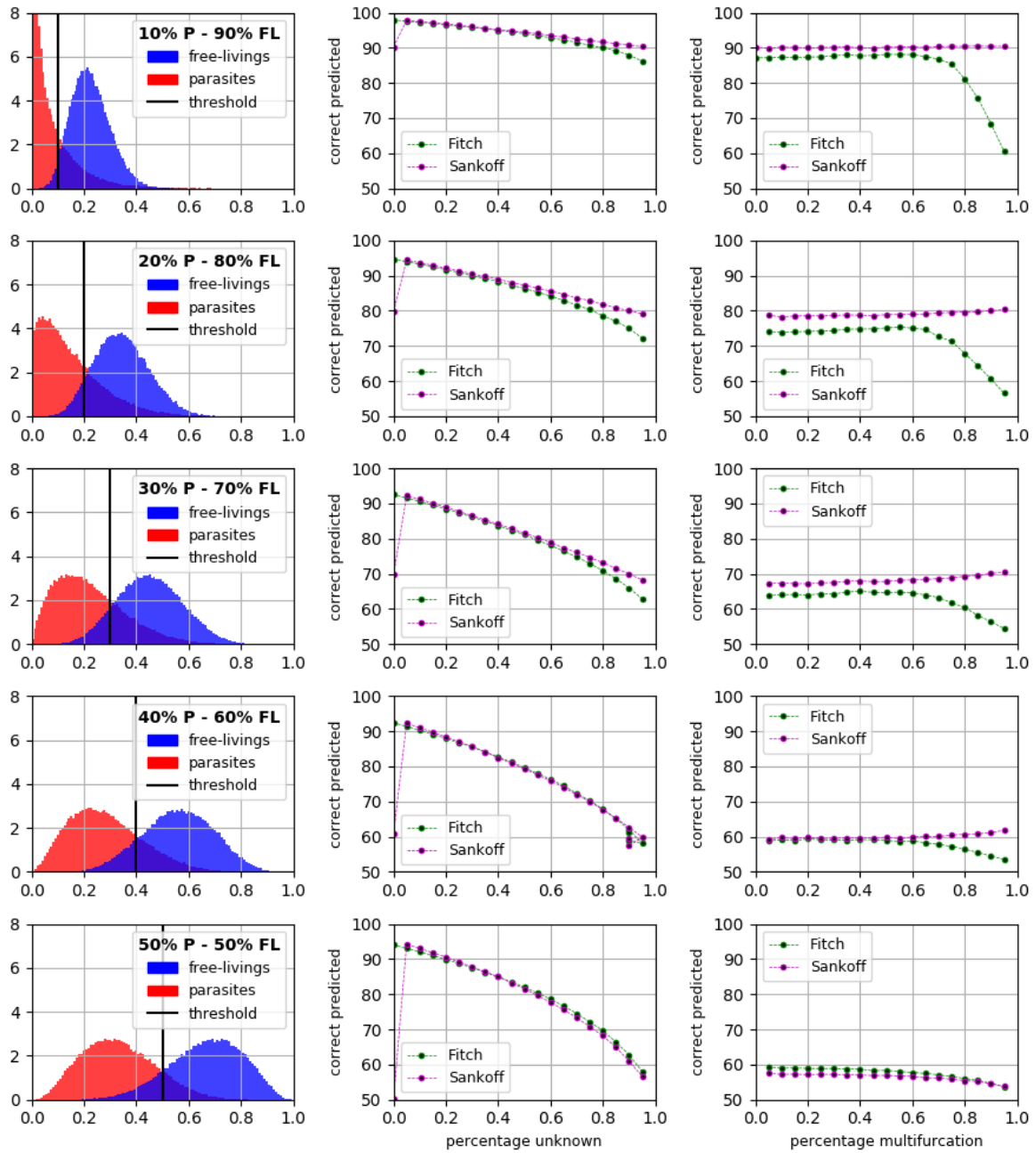


Figure 3.2: Influence of unknown data to prediction

In the end, Sankoff is in most cases the more accurate algorithm and was therefore used for our prediction of the real data.

3.3 Results of the real data analysis created with Sankoff

3.3.1 Biological view

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

In Table 3.5 we compare the given states with the predicted ones.

There are several factors that play a role here, and in part, crystallize in these examples.

An important factor here is that the credibility of the results with the accuracy of the input data, i. the data of GloBI, stands and falls. 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. Positive in the sense that the majority have the opposite state.

Phylum	# nodes	original states		final states			
		FL	P	0 (FL)	0.3	0.5	1 (P)
Chordata	91785	10451 99.83 %	18 0.49 %	91759 99.97 %	0	0	26 0.03 %
Nematoda	30127	21 0.63 %	3289 99.37 %	1604 5.32 %	142 0.47 %	1196 3.97 %	27185 90.23 %
Platyhelminthes	22683	7 0.1 %	7086 99.9 %	175 0.77 %	0	0	22508 99.23 %
Apicomplexa	1863	1 0.39 %	255 99.61 %	1 0.05 %	0	0	1862 99.95 %

Table 3.5: Phylum (leaf nodes)

In contrast to the other phyla examined, the phylogeny is more pronounced (less multifurcation) (see Table 3.1). This results in a weaker spread of errors. This is reflected in the results from the table 3.5. 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).

TODO: A few punctual tests give us the following:

We mapped the few parasitic species in a rough taxonomy (see Figure 3.3):

Known parasitic birds belong to the order Sauria. Here we know from Rothschild here there are breeding parasites, like the cuckoo and clepto-parasites as the skuas [26]. 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 seems to be a paper from the GloBI concluding: 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 [27]. 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 had more state information for the leaf nodes compared to the other considered subtrees, see Table 3.1. We start with 0.1 % free-livings and predicted

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

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

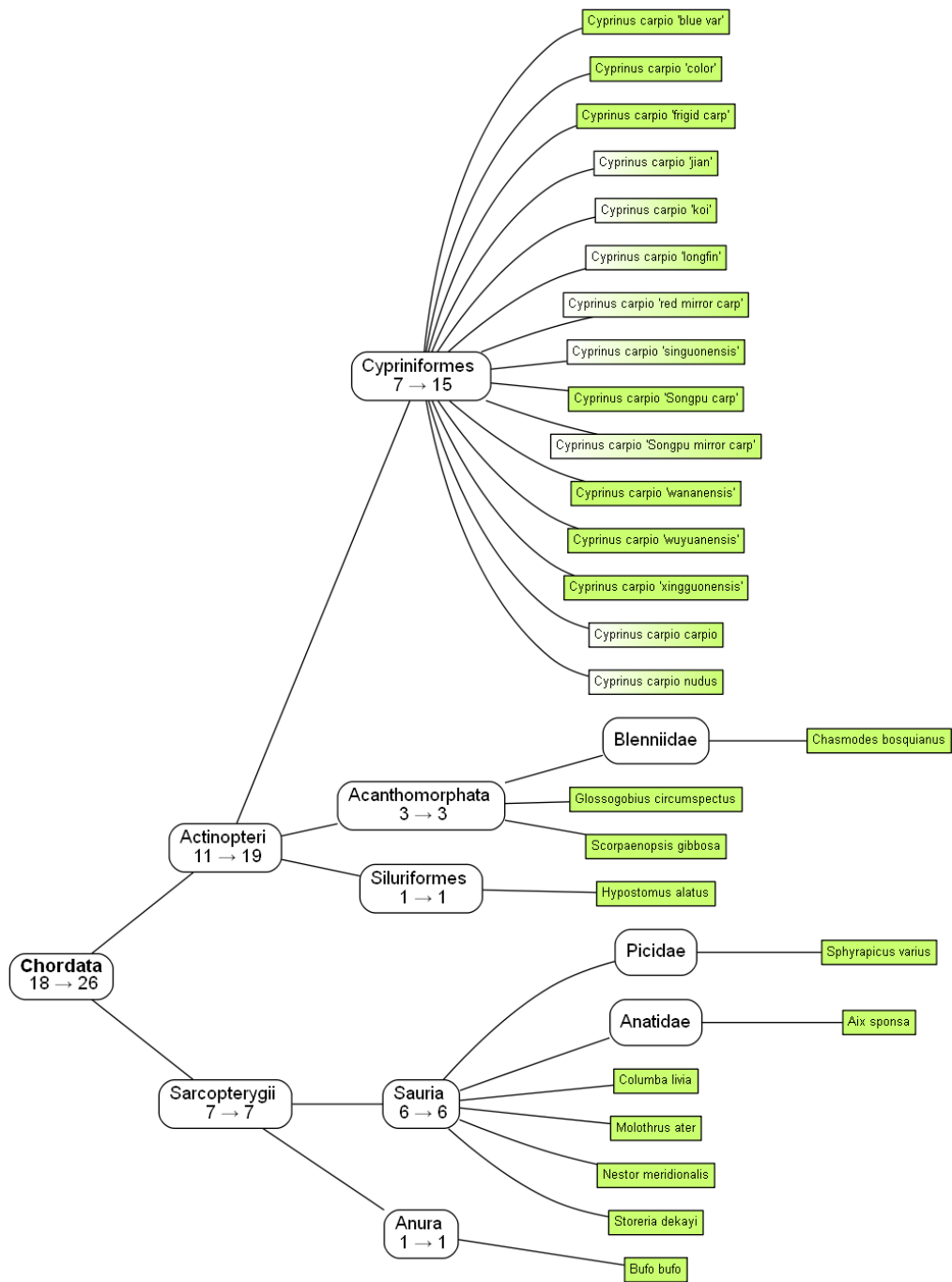


Figure 3.3: Parasites of Phylum: Chordata

Input parasites (green) → predicted parasites (white → green)

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 [28] and speaks of at least seven independently arose parasitism [29]. In a recent article Blaxter identifies 18 origins [28] in Nematoda.

The problem at this point, however, is obvious: Hallan speaks of the fact that only 23,00 species were described by the Nematoda but one assumes 1 million or more³.

TODO: Link ist nicht erreichbar! The parasites have been much more studied and thus we start with only 0.63 % free-living species. Against such a shifted data situation, the algorithm is almost powerless. And yet the percentage has increased.

TODO: Im Folgenden folgen 3 weitere ähnliche tabellen. Einmal eine ähnliche Tabelle inklusive interner Knoten 3.6, zweimal die Übersicht über die Kingdoms Blattknoten 3.7 bzw interne Knoten 3.8. Welche nehmen wir? Rest Appendix oder ganz raus?

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 3.6: Phylum (inkl internal nodes)

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	75446	45	529	13426	220	24082	0	7792	5302	0	24493
Fungi	31457	577	2983	38520	0	0	0	5723	0	0	266463
Chloroplastida	416478	3519	77	410795	0	0	0	4182	0	0	1501
Metazoa	1491012	30758	22373	1328135	0	0	930	25535	4423	1665	130324

Table 3.7: Kingdom (leaf nodes)

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

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 3.8: Kingdom (inkl internal nodes)

3.3.2 Origins and Losses

Weinstein and Kuris have been searching for origins of parasitism in Animalia [4]. 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. So how do **you** count? Our solution was, to round these values. We have to say that we round 0.5 to 0.

In Table 3.9 we can see, that we found some more origins than Weinstein and on top of that some losses.

Lets have a look at 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 are looking fine too. 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 rootnode 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 [29].

Domain / Kingdom / Phylum / Class	# internal nodes	# leaf	Rootnode state	without and # origins (FL -> P)	with rounding # losses (P -> FL)
Eukaryota	241974	2293463	1.0 P	415 462	363 369
Metazoa	179944	1491012	0.5	294 321	123 129
Fungi	9534	314571	0.5	80 97	222 222
Chloroplastida	43486	412434	0.0 FL	40 42	2 2
Arthropoda	120479	1198981	0.0 FL	260 281	102 108
Apicomplexa	239	1863	1.0 P	0 0	1 1
Nematoda	3437	30127	1.0 P	0 2	11 11
Chordata	30761	91785	0.0 FL	12 12	1 1
Platyhelminthes	4459	22683	1.0 P	0 0	5 5
Insecta	91256	989572	0.0 FL	234 245	77 77

Table 3.9: Origins and losses

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.

possible tags:	0,	0.333,	0.4,	0.5,	0.667,	0.75,	1
rounded to:	0	0	0	0	1	1	1

3.3.3 Cross evaluation - leave 100 out

We ran the castor algorithm 100 times with leaving 100 randomized free-living or parasitic species out of the input data to see how stable our result is. Of these 10,000 nodes, 9,238

were unique. Of that, we predicted 9060 ($\approx 98.17\%$) correctly and 169 ($\approx 1.82\%$) wrongly, with duplicate draws always having the same prediction.

What is the best way to model this data? We again tested the influence of the taxa and the depth of leaf nodes and calculated the BICs (Table: 3.11).

Model / Taxa	Kingdom	Phylum	Class	Order
correct predicted \sim taxa	117877	111466	XXXXX	XXXXX
correct predicted \sim taxa + depth	117703	XXXXX	XXXXX	XXXXX
correct predicted \sim taxa * depth	117592	XXXXX	XXXXX	XXXXX

Table 3.10: Residuals of cross validation prediction

Model / Taxa	Kingdom	Phylum	Class	Order
correct predicted \sim taxa	117936	112242	111733	XXXXX
correct predicted \sim taxa + depth	117776	XXXXX	XXXXX	XXXXX
correct predicted \sim taxa * depth	XXXXX	XXXXX	XXXXX	XXXXX

Table 3.11: BICs of cross validation prediction

What could happen by removing a parasite or free-living of the list?

- It could be a specie, which don't exist in the tree leaf nodes. -> no effect
- It could be a specie, which exists in both lists. -> If it was a parasite, it is now free-living, because we prefer parasites. Otherwise we have no effect again. (1053 are possible)
- Normal case: We loose information, because its a specie in our tree and we change it to a leave node with no information.

Influence on the rest of the data:

3.4 Effects of Taxa in the different models

The comparisons of the effects of the taxa can be found in Table 3.14 and showed ...

		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
changed tag		0	0	0	0	0
lost	all tags	100	100	100	0	0
	FL tags	44	66	57.25	19.50	4.42
	P tags	34	56	42.75	19.50	4.42

Table 3.12: Statistics to Cross validation

		min	max	mean	variance
distance	all	1	2	1.33	0.33
		0	3587.70	217.94	273760.68
	leaf nodes	1	2	1.33	0.33
		0	3021.12	202.57	209274.86
	internal nodes	0	0	0.00	0.00
		0	566.58	15.37	5684.00
lost	FL tags	44	49	46.67	6.33
		51	66	57.95	13.47
	P tags	51	56	53.33	6.33
		34	49	42.05	13.47

Table 3.13: Statistics to Cross validation

- Less free-livings - 3 examples
- Less parasites - 64 examples

Taxa	Model / Effects	min	max	mean	median
Kingdom	globi ~ taxa	0,01	0,04	0,02	0,01
	globi ~ taxa + depth	0,01	0,03	0,02	0,01
	globi ~ taxa * depth	0,00	0,03	0,01	0,01
Phylum	globi ~ taxa	0,17	393501,80	32208,79	6149,78
	globi ~ taxa + depth	0,32	567010,90	55149,54	10783,18
	globi ~ taxa * depth	0,00	1000000,00	225375,33	2511,58

Table 3.14: Effects of Taxa in models for unknown data

globi ~ taxa * depth: NOTE: kingdom is not a high-order term in the model

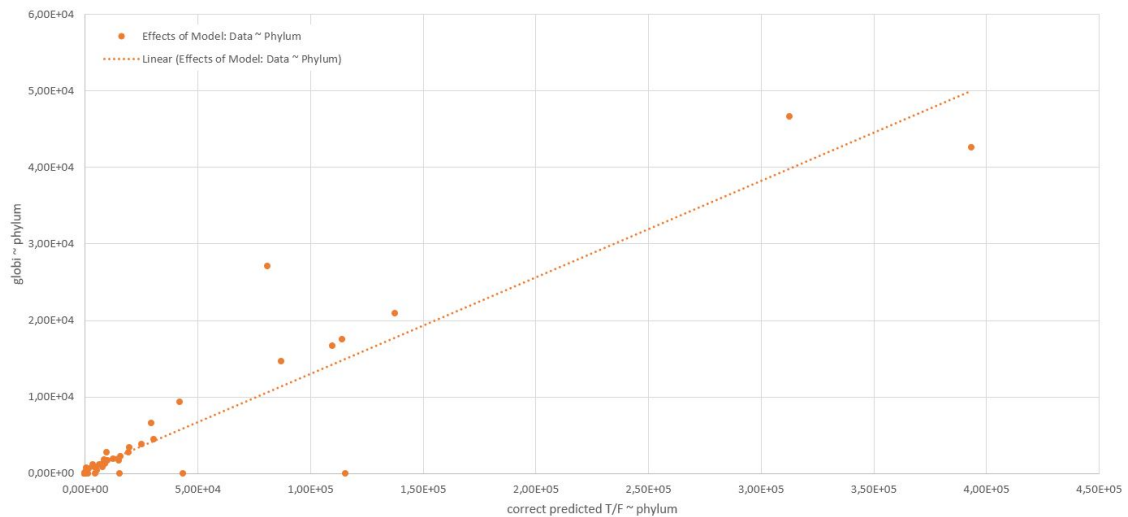


Figure 3.4: Effects of Model: Data ~ Phylum

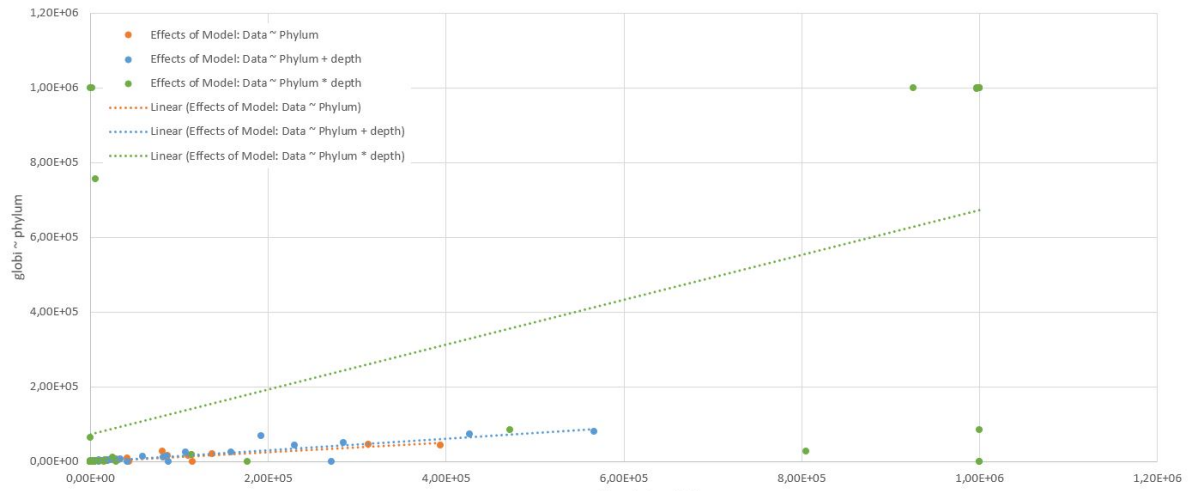


Figure 3.5: Effects of 3 Models: Data \sim Phylum (+/* depth)

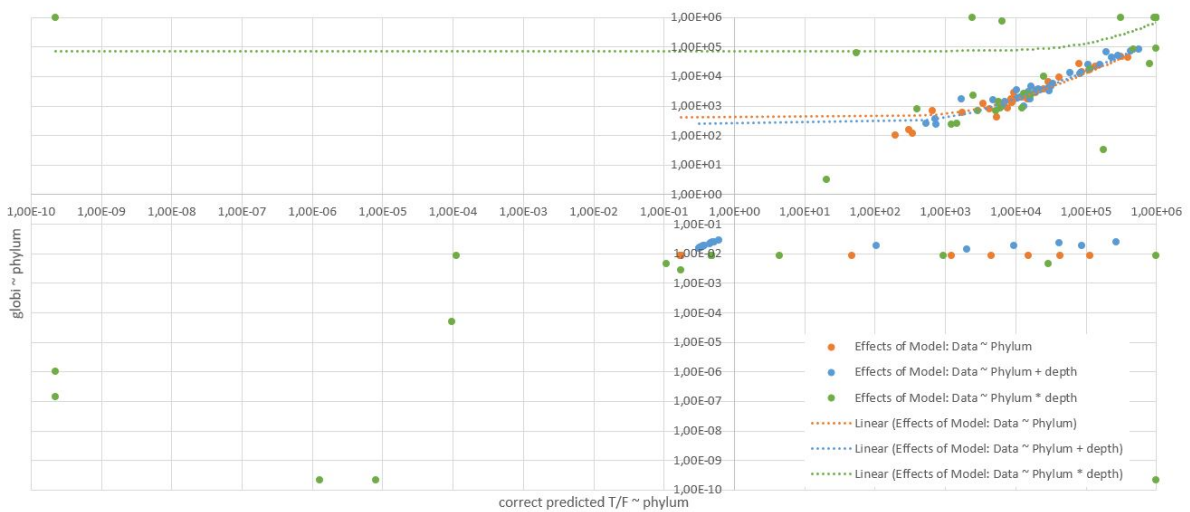


Figure 3.6: Effects of 3 Models: Data \sim Phylum (+/* depth)
both axis in log scale

4 Discussion

Fehlerquote der Daten an sich?

Wie gut ist unsere Datenlage? 3 mio knoten, 1.8 named species (leaf nodes), 200.000 leaf nodes mit Information.

Welche Teile des Baumes sind gut, an welchen muss noch viel geforscht werden.

Wieviele Origins haben wir gefunden, was bedeutet diese Zahl?

Es gibt noch ungenutzte information in GloBI.

Wir können GloBI verbessern ... Wir haben GloBI auf die gefundenen Abweichungen aufmerksam gemacht... Fehler werden durch die prediction eventuell verstärkt...

4.1 Simulation

For fixed calculations we assume a distribution of 60% free-living to 40% parasites, 95% missing data and a multifurcation rate of 95%. How well does our simulation approach the real data situation?

There are some points to discuss:

- How close is the randomized binary tree to a true phylogeny?
- The distribution of parasites to free-ranging species is a pure assumption. From different sources about 40% parasites are estimated, are these also beta-distributed?
- How well do the transition probabilities match parasitism? Depending on the type of parasitism, this will certainly look different. In addition, it can be assumed that the probability for losses is lower than for origins.

- The equal distribution of the multifurcation is a blank assumption. It is very likely to be higher towards the root node because there is less information back in time. On the other hand, there are many studies that have studied various species without considering phylogeny. Which also creates a high number of children of a node directly at the leaf nodes.

In order to be able to observe these problems, we have programmed our simulation according to the parameters. This allowed us to estimate the influence of some of these parameters.

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5 Appendices

5.1 Methods overview

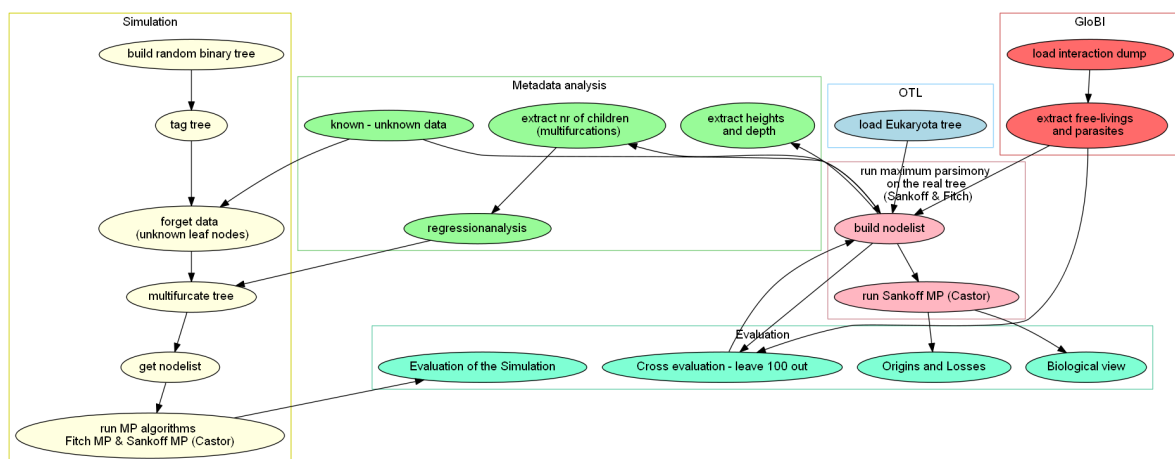


Figure 5.1: Big overview of the whole Workflow

5.2 OTL analysis

5.2.1 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

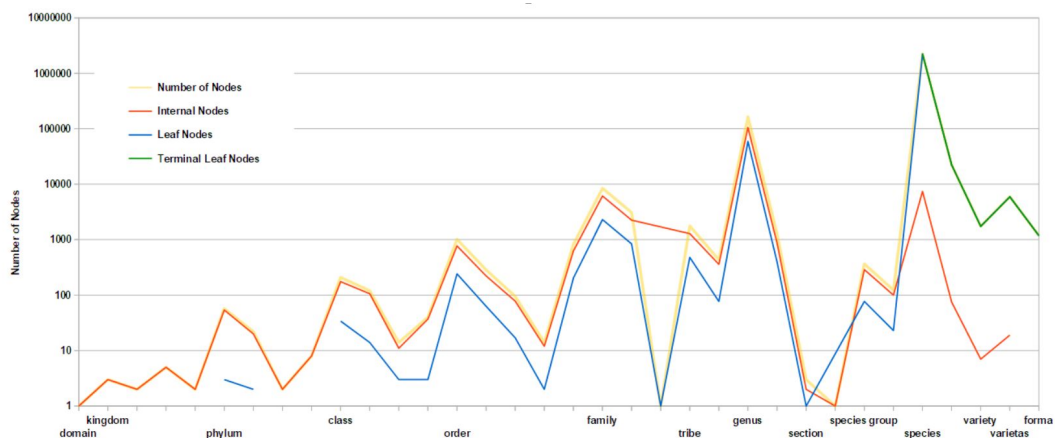


Figure 5.2: Distribution of Nodes in Rank-Categories

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 were 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 5.2.

... (see Table 5.2)

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 5.2).

5.3 Missing leaf state modelling - Residual tables

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 5.1: 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 5.2: TODO: ...

Model / Taxa	Kingdom	Phylum	Class
multifurc ~ taxa	545740	499227	482265
multifurc ~ taxa + depth	544789	493017	478998
multifurc ~ taxa * depth	544062	488366	476382

Table 5.3: Residuals of unknown information