An analysis of maximum parsimony algorithms to predict parasitism in Eukaryota

using a large multiurcated phylogenetic synthesis tree

Abstract

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

This paper is about the analysis of ancestral state reconstruction algorithms for non-binary trees, applied to the currently largest phylogeny synthesis tree of Open Tree Of Life, with the application of prediciton of parasitism.

Anmerkung: Mein Vorschlag einer Gliederung (jeweils ca. ein Absatz) (Bernhard)

i) Motivation:

- Was ist das große Ziel?
  Das Ziel dieser Arbeit ist die Anwendung von maximum parsiomony algorithmen auf nicht binäre Bäume und auf sehr große Datensätze. Insbesondere auf das Beispiel 'Entstehung des Parasitismus' im ganzen Eukaryotischen Tree of Life.
- Was soll erreicht werden?
  Wir wollen vorhandene Algorithm (Sankoff/castor [3]) auf diese Aufgabenstellung hin testen und ihre Vorhersagekraft abschätzen. Außerdem wollen wir den Fitch algorithmus für binäre Bäume auf unser Problem erweitern und mit dem Sankoff Algorithmus vergleichen.
- Warum ist das relevant? Was könnte man dann tun?
  predict states of species...
  TODO: !!!

ii) Hintergrund:

- Was gab es in dieser Richtung bereits als ganze Ansätze oder wenn nicht, warum nicht? Woran ist es bisher gescheitert?
  Bisher wurden vorallem Algorithmen für das binäre Problem entwickelt, da man

wesentlich kleinere Teilbäume betrachtet hat, von welchen man auch alle Aufspaltungen kennt. Durch die Entwicklung von OTL, eines gesamten Baum des Lebens, ergibt sich das Problem, dass dieser bei weitem nicht binär ist.

Researchers of the phylogenies have been dealt with the ancenstral state reconstruction in the 60s. The first methods were only brute force TODO: Quelle, siehe Fitch: Camin and Sokal 1965 . Next came a set of parsimony algorithms such as: Fitch-parsimony [5], Wagner-parsimony [6] ... TODO: weitere? .
With more and more data, there is now the possibility to use more information to calculate the probabilities of the ancestral states. In addition to the states of the leafs, algorithms could also use branch lengths. The likelihood based algorithms came more in interest.
Our focus came with another 'data extension'. We wanted to work with the biggest phylogenetic tree that exists at this moment, which goes over all observed species. For most TODO: most? species there is no phylogeny, but only a taxonomic classification.

- Welche Grundlagen sind notwendig:
  - open tree of life: Was ist das, warum relevant und überlegen als reine Ansätze?
    TODO: !!!

    So the biggest 'phylogenetic tree' is a synthesis of phylogenetic trees filled with a taxonomic tree given by Open Tree of Life [1]. This tree is not binary and therefore the developed algorithms are not directly applicable.

  - Algorithmen: Was gibt es? Ruhig ausführlicher als hier bereits und vor allem auch nach einer Darstellung am Ende ableiten, was für uns relevant ist. Also beschreiben, wie Methode a, b, c funktionieren und dann abwägen, was daher für Dich am relevantesten ist.
    TODO: !!!
    Anmerkung: GloBI und OTL in der Einleitung vorstellen. (Emanuel)

iii) Outlook/Structure of this work

In this work, we have looked at the algorithms that are generally suited to our data, to develop them further for the not binary case, and finally to compare their usability with our sythesis tree.
We have decided to consider only parsimony algorithms since we have no information on branch lengths and no other additional information like different transition probabilities of our states.

In den Bacteria und Archaea wurde das mal allgemein für binary traits gemacht: "A total 90% of all binary traits described molecular functions, specifically the presence or absence of a gene or a set of gene involved in biochemical pathways." [7]
Unterschied bei uns ist parasitismus komplexer.. da sind meist nicht einzelne Gene zuständig..

1.1 Definitions

- Parasit - Freilebend
- Multifurkation - binär
- height (min, max, mean), depth of a tree/node (Distanz zur Wurzel vs distanz zum Blatt)
- maximum parsimony
- OTL, OTT, GloBI

Parasite
Since we use Globi to classify species as parasitic or free-living, we use their definition of parasitism. In GloBi, ontobee definitions are used. 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/...

2 Methods

As initiated, we would like to apply a maximum parsimony algorithm to the entire 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, we decided to use a simulation to decide how to evaluate the existing algorithms and possibly adapt them to our given problem.
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.

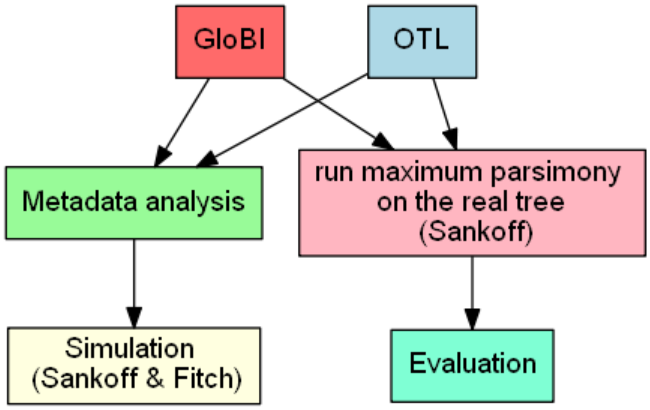


Figure 2.1: Workflow

The coming sections are thus subdivided into the following topics:
TODO: or: The resulting procedure is as follows:
(1) Get the real tree and real data for the leaf nodes → OTL, GloBI databases. (2) Get metadata of these for a realisitc the simulation. (3) Build and run the simulation. (4) Evaluation of parameters for the simulation and the real problem. (5) Run the resulted algorithm on the original data. (6) Evalute and interprete results. → Origins etc...

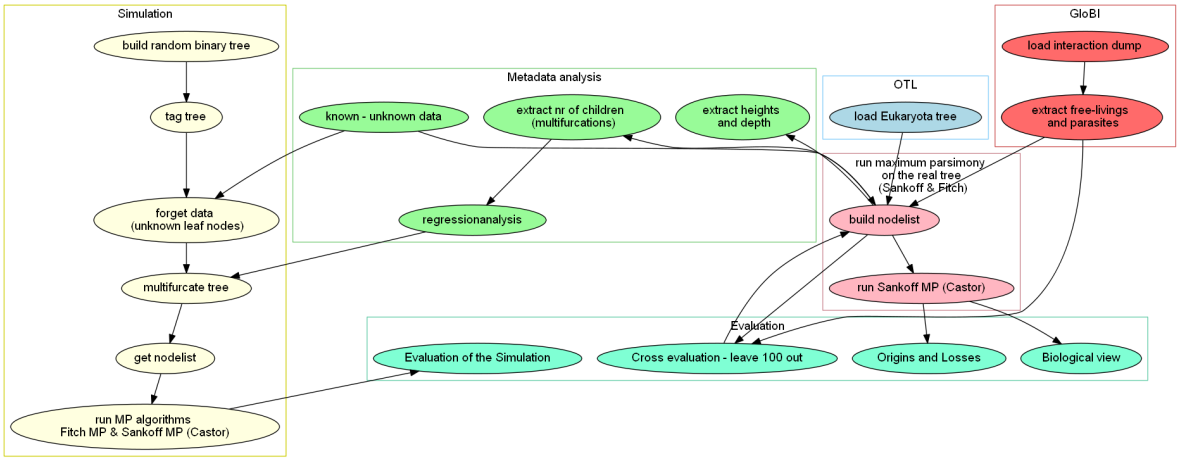


Figure 2.2: Big overview of the whole Workflow

2.1 Get data - Properties of real Data

For our research we need two types of data: a tree and information about the states.
For the tree we decided to use Open Tree of Life (OTL).
For the state information, we decided to use the Global biotic interaction database (GloBI).

2.1.1 OTL

For our project we looked for a large database for phylogenetic trees and also for a taxonomic tree. Since we run our algorithm on the phylogenetic tree, and for the evaluation and other properties the taxonomy provides us with much more information.
OTL gives us both. 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) [8], NCBI (National Center for Biotechnology Information) [9], WORMS (World Register of Marine Species) [10], GBIF (Global Biodiversity Information Facility) [11], OTT (OpenTreeOfLife-Taxonomy) [1].
TODO: Marius: "Every dataset has it's own characteristics and downsides. 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.
We choosed the taxonomy from OpenTreeOfLife because it's including most of the known taxonomies and got synthesised by preffering taxonomies that match with available phylogenetic data. At the same time the team from OTL preferred a maximum number of species [1]. This is resulting in somekind of hybrid between taxonomy and phylogeny."

We took a closer look at 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 5.1.

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) [12], Webs on the Web [13], Animal Diversity Web [14] and ecoweb [15]. 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). We appointed some interactions[1] , where we know from the biological perspective that the species source or target has to be a parasite or a free-living species. 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

[1] https://github.com/jhpoelen/eol-globi-data/.../InteractType.java

- parasite target: hasParasite, hasPathogen

We build two lists: parasites and free-livings, and add the source or targets of an interaction to these.

2.2 Metadata analysis

In order to generate the most realistic simulation, influencing parameters were 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

We tested the influence of these parameters 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 [16] 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 were equated. However, the used castor package [3] offers the possibility to enter different transition probabilities.

In the simulation, we chose two beta distributions and a threshold that indicates the change between states.
Different thresholds with different beta distributions were 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 (TODO: see results simulation, ref...). Figure 2.3 shows one example of these.

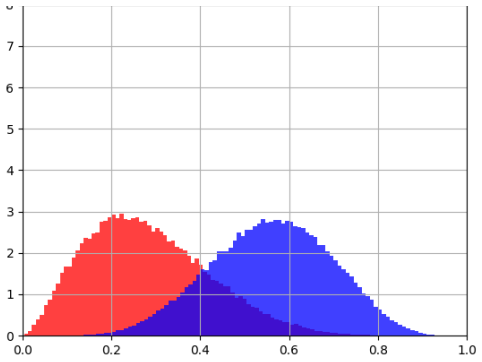


Figure 2.3: 60% Free-living - 40% Parasites red: parasites, blue: free-living, e threshold is at 0.4

TODO: Plot neu erstellen: Achsenbeschriftung, threshold

2.2.2 Multifurcation

We have a very high multifurcation rate (see results section 23). Therefore, the impact of multifurcations on our research question has been investigated.
Generalized Linear Models with poisson regression were compared according to their residuals and BIC.
Anmerkung: Nested models were compared using likelihood ratio tests, models using different predictors were compared according to their deviance and AIC. (Emanuel)
TODO: Einfluss auf die Vorhersage des Castor und Fitch Algorithmus (Simulation)

2.3 Simulation

There are various possibilities of ancestral state reconstruction. The simulation compared different algorithms.
On the one hand different implementations of the Fitch maximum parsimony were compared and on the other hand the best of them with the implementation of the sankoff algorithm of the castor package [3].

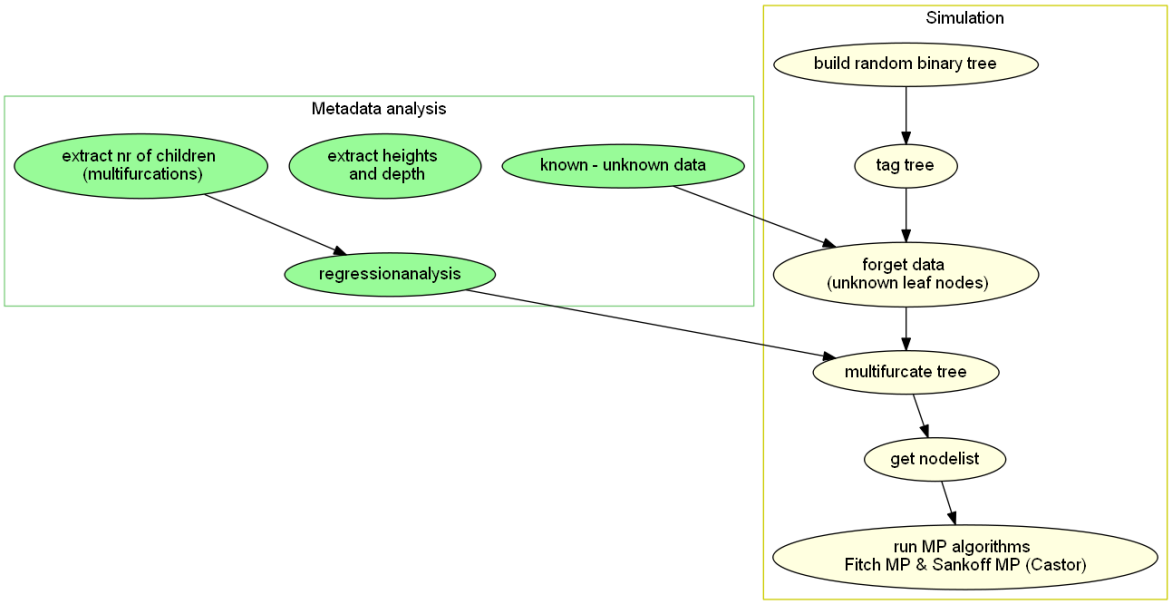


Figure 2.4: Course of the simulation with influence of the metadata analysis from the real data.

Figure 2.4 shows the course of the simulation. The individual steps are explained in the following subsections.

TODO: Evaluation - compare trees (distances)

2.3.1 random binary tree

You need a tree 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 were 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 were used [17]. They offer a randomized function which returns a BaseTree[2].
TODO: ref in die discussion über die randomized function? Diskutieren wir das?

2.3.2 simulating states and transitions between them

The next step is to simulate the states of the nodes using the transitions. Again, we simulate fully known states and then 'forget' everything but a few in the leaf nodes so that you can later compare the reconstruction with the origin.
The root node (ancestor of all subsequent species) is (of course) free-living. That means it will start in the free-living beta distribution. 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.
To ensure that the parameter of the binomial distribution is restricted to the [0,1] interval, we model it with a beta distribution as in Figure 2.3.

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 $\rightarrow$ 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.

[2]https://github.com/biopython/biopython/blob/master/Bio/Phylo/BaseTree.py

Different percentages of forgetting the information were 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. We simulate this multifurcation of the tree by TODO: ... .

On the one hand, the strength of multifunctionation plays a role, as does the distribution. Modeling was done as explained in TODO: sec x .

TODO: bisher ohne Begründung:!!! we decided to use a $\frac{1}{x}$ distribution, where x is the depth of a node. This means, how deaper we are, how less information we have.
We traverse through the tree and pick a random number between 0 and 1. If random number is smaller as our limit ($\frac{1}{x}$), than we forget the node and hang every child to the father node of the current node.
Anmerkung: poisson process → fit that distribution, include depth as a predictor, see if significant (Emar

```
  def get_non_binary_tree(subtree, nodelist):
    i = 0
    while i != len(subtree.clades):
      if subtree.clades[i].is_terminal():
# is leaf node?
        i += 1
      else:
        element = Helpers.find_element_in_nodelist(subtree.clades[i].name
        limit = get_limit(element[1])
        new_random = random.uniform()
# choose if we want to delete ourselve
        if new_random < limit:
# or new_random < 0.9:
          subtree.clades += subtree.clades[i].clades
# add children
```

```
            del subtree.clades[i]
# delete internal node
        else:
# if we don't deleted ourselve go on with children
            get_non_binary_tree(subtree.clades[i], nodelist)
# otherwise the children are in the current clade array
            i += 1
    return

    def get_limit(depth):
      limit = 1 - 1 / ((depth + 3) / 4)
      if limit < 0.1:
        limit = 0.1
      return limit
```

Wir lassen das Limit nicht beliebig klein werden, sondern beschränken es auf 0.1.

With this loss of information the reconstruction can start.

2.4 ancestral state reconstruction methods

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 [18].

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 divergece times (branch length) into account. Since we have no development times 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 for dollo parsimony. Again, this problem is irrelevant for us, because you can only work with generalized models in the analysis of the entire Eukaryota tree.

Since the simpler parsimony methods are sufficient for our case, we have decided for them.
TODO: In the following we introduce ... there are these methods .. which are important for us ...

2.4.1 Fitch maximum parsimony

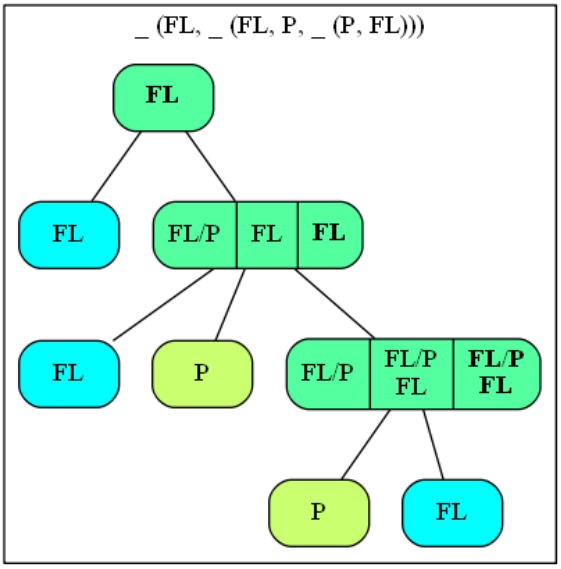
Described from [COO98] + others ... - implemented for multifurcating trees

Fitch algorithm for binary trees:

Der Baum hat die folgende Struktur: Alle inneren Knoten sind leer. In den Blattknoten befindet sich entweder das Tag FL oder P, oder deren Vereinigung, wenn es sich um einen unknown node handelt.

Der Fitch Algorithmus ist aufgeteilt in drei Schritte, in welchen man jeweils durch den Baum traversiert. Schritt 1 beginnt von de Blättern aus, da sich dort zu Beginn die einzige Information befindet. Für jeden Knoten gilt, wenn seine Kinder schon Information enthalten, dann bilde die Schnittmenge der states und schreibe diese als Information in den aktuellen Knoten. Ist die Schnittmenge leer, dann schreibe die Vereinigung aller möglichen states in den Knoten. Für alle Kinder, die noch keine Information haben, führe diesen Schritt erst für diese aus. Schritt zwei geht von den Kindern der Wurzel bis zu den Vätern der Blätter. Jeder Knoten bekommt einen zweiten Tag, der sich aus der Vereinigung des states des Vaterknoten

Figure 2.5: bla



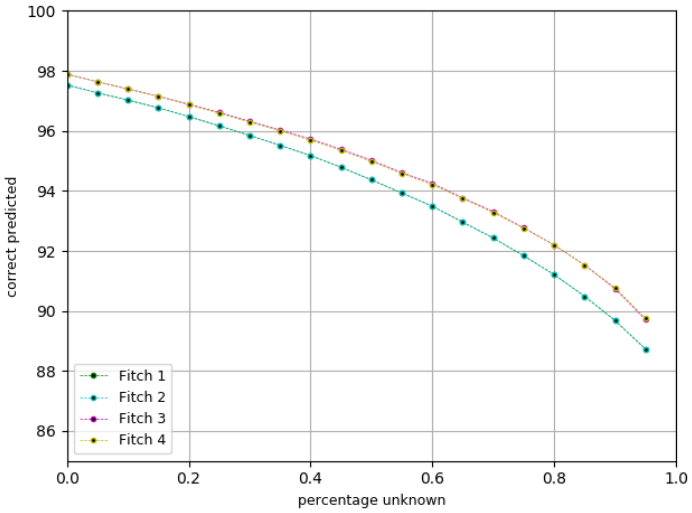
und der Geschwisterknoten zusammensetzt. Ist diese leer, bekommt der Knoten wieder die Vereinigung aller states, also $\{FL, P\}$ als Tag.

Hier gibt es einige Möglichkeiten, wie dieser Schritt genau aussieht. 1. Version: Es wird nur der erste Tag vom Vaterknoten genutzt. Außerdem wird von den Geschwisterknoten zuerst der Schnitt gebildet, und danach vom Ergebnis nochmal mit dem Vaterknoten zusammen. (Immer wenn der Schnitt leer ist, ist das Ergebnis die Vereinigung aller states, also $\{FL, P\}$. Auch im folgenden...) 2. Version: Es wird nur der erste Tag vom Vaterknoten genutzt. Er wird zusammen mit den Geschwisterstates genommen und direkt ein Schnitt aller Mengen gebildet. 3. Version: Es werden alle vorherigen states vom Vater genutzt und von diesen ein Schnitt gebildet. Das selbe gilt für die Geschwisterstates. Und dann wird ein dritter Schnitt zwischend en Ergebnissen gebildet. 4. Version: Es werden alle states genutzt und direkt in einem Schnitt zusammengenommen.

Der Finale Schritt traversiert nochmal über den Baum und Bildet aus den zwei states pro Knoten einen finalen Tag, indem wieder der Schnitt der beiden states das Ergebnis ist.

Ich habe diese Versionen mit 100 Bäumen mit 10000 Blattknoten und der Verteilung 60% FL zu 40% P simuliert. Bei 90 % unbekannten Knoten lag Version 1 zu

Figure 2.6: bla



89.67 %, Version 2 zu 89.67 %, Version 3 zu 90.72 % und Version 4 zu 90.74 % richtig.

How to extend Fitch for multifunction?:

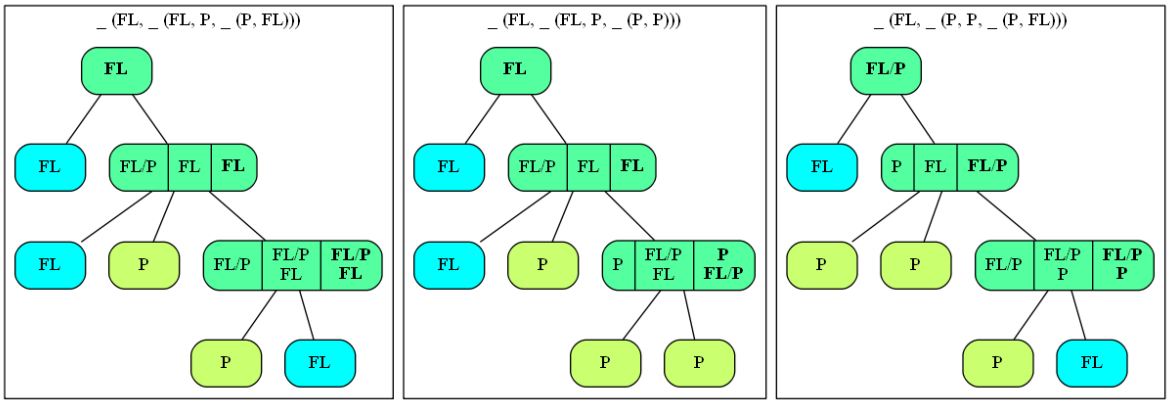
Sankoff

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

2.5 real data analysis

- Import tree
- Import interactions
- run castor algorithm / and others?
- interprete results (cross validation - leave 100 out)

Figure 2.7: bla



2.6 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

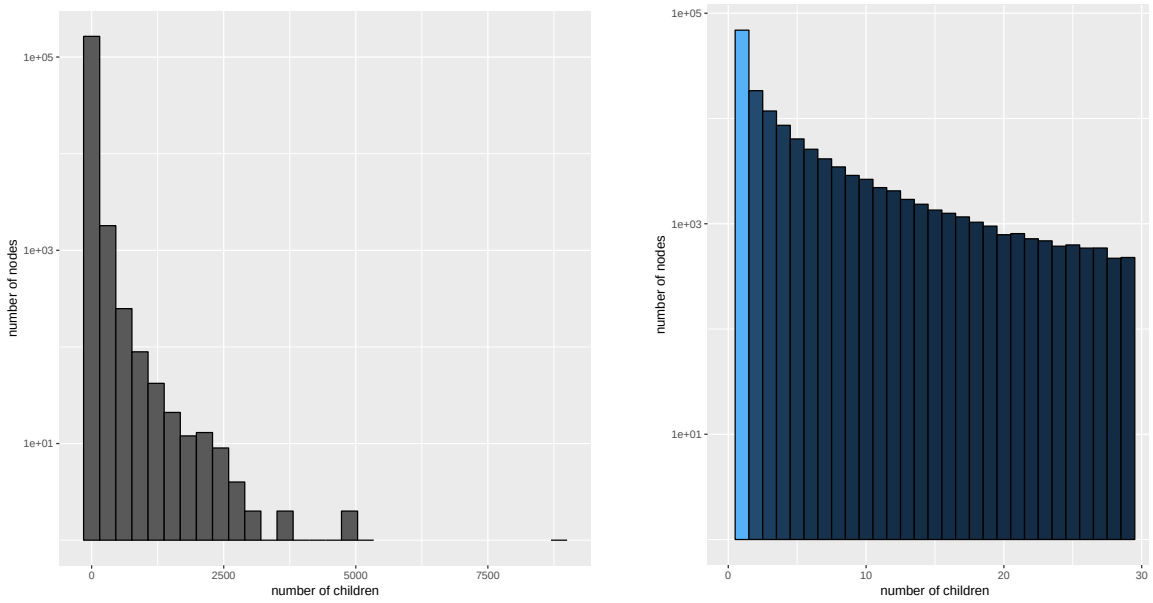
3.1.1 Taxa

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, Hans says that one distinguishes between seven and nine kingdoms [19].
In section 41 of the appendix you can find a list of all phyla.

3.2 Multifurcation

One property of the tree is its ridge of multifurcation. 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.



(a) Histogram with automatic binwidth.

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

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

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 discribes 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.

Data artifacts

At this point we also found out that there are some nodes with only one child node (55700 nodes).
These are both, the most nodes are right in front of a leaf, as well as some nodes are deep

in the tree (3956 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

3.2.1 Poisson regression

glm (generalized linear model) analysis:

The intercept is $2.821 > 0 \Rightarrow$ there is a multifunction. (Intercept: Stärke der Multifurcation)
Comparing the different kingdoms, we find that multifunctionality is greater in Fungi than in Chloroplastida than in Metazoa:

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

Wir haben außerdem drei komplexitätsstärken von Modellen verglichen bezüglich der höhe und tiefe des Baums mit dem folgenden Deviance Table:

* Residuals: Fehler - wieviele Werte sind nicht gut modelliert. (umso kleiner umso besser - grün)

Interpretation: Die Multifurkation ist sehr ungleich verteilt. Daher ist die vorhersage umso genauer umso kleinere Subtrees wir betrachten. ...

Because of the difference in the complexity of the models, we compared their BICs:

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 3.1: Residuals...

Model / Taxa	Kingdom	Phylum	Class	Order
multifurc ~ taxa	8273333	7937828	7842157	7644249
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 3.2: BIC...

3.3 Unknown leaf nodes

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 $\rightarrow$ unknown nodes 2194794 ($\approx 95.7\%$).
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.
TODO: With this only $\approx 4.3\%$ information in our leaf nodes are ...
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 dimensions.

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

Table 3.3: Residuals of unknown information

Model / Taxa	Kingdom	Phylum	Class
multifurc ~ taxa	545799	500004	485121
multifurc ~ taxa + depth	544862	493808	481869
multifurc ~ taxa * depth	544179	489845	481494

Table 3.4: BICs of unknown information

TODO: Taxa like order or family were too expensive to calculate...

3.3.1 Results of simulation / Influence of different parameters

3.4 Results of castor

3.4.1 Biological view

TODO: Castor replaces originaltags with finaltags. There are 82 originaltags != finaltag.

We picked a few phyla to evaluate the results from the biological point of view.
Table 3.5 shows some known phyla: Chordata, Nematoda, Platyhelminthes and Apicomplexa.
Since GloBI is not perfect, all examples contain a few bugs. As known the Chordata are full

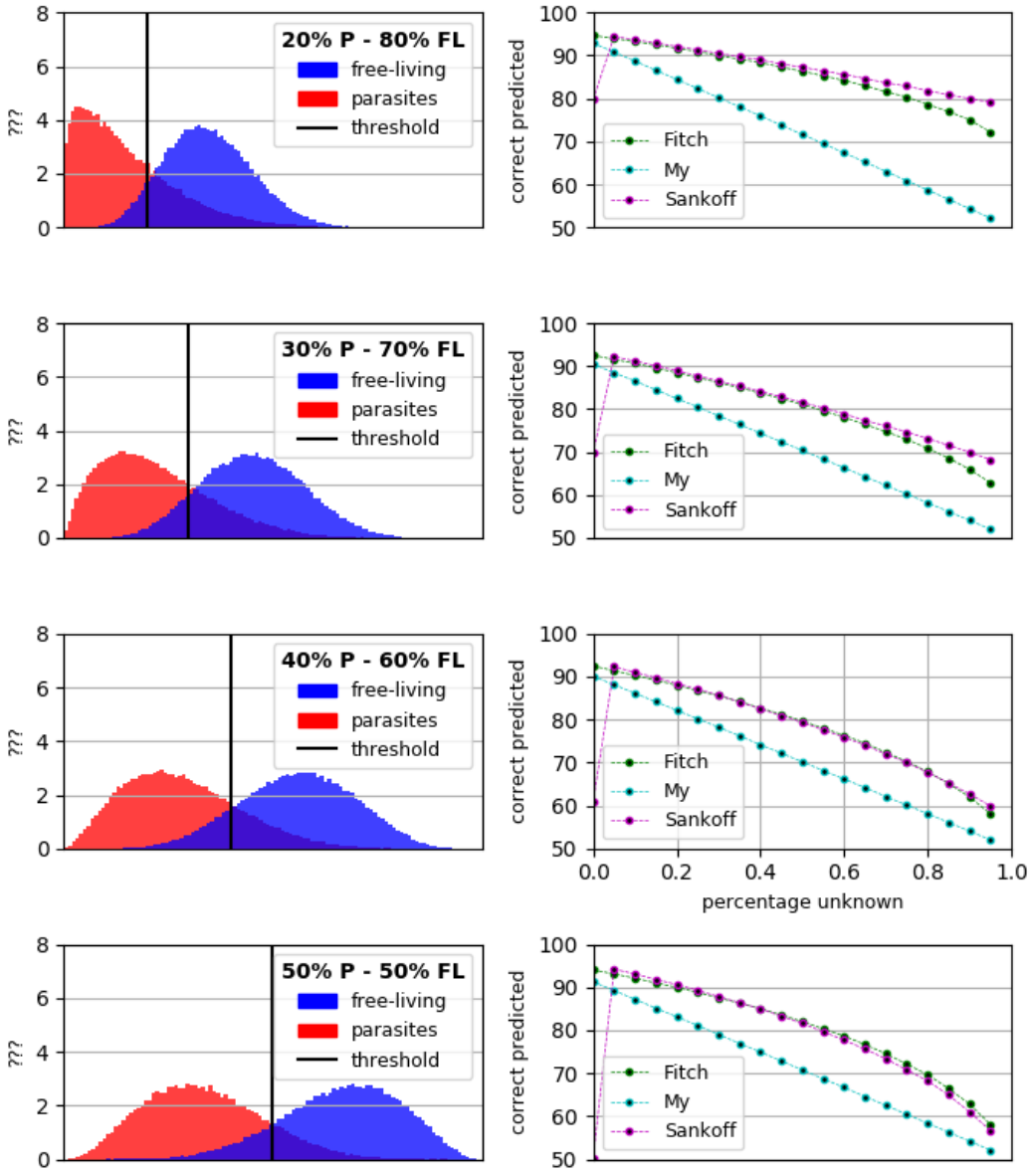


Figure 3.2: Influence of unknown data to prediction

of free-living species and there are only a few parasites. Among the birds are some breeding parasites (brood parasitism) like the cuckoo and clepto-parasites as the skuas [20]. The algorithm reflects this. We started with 99.83% free-living species and predicted 99.94% species as parasites TODO: (inklusive all known nodes) . Only 0.06% were predicted as parasites.

TODO: We found the cuckoo and some skuas...?

Same observation but with less free-livings is the Apicomplexa Phylum. Here we have only a few free-livings TODO: ref einfügen . And as we see, we had good start data and predicted 00.95% as parasites.

For the Platyhelminthes the literature says that there are mostly all Platyhelminthes parasites TODO: ref einfügen . But at the end we predicted 4.18% as free-living. The class Seriata is the reason for the most of free-livings in this phylum. These are partly free-living flatworms, so the prediction looks right.

TODO: Wiki:

*The Seriata are an order of turbellarian flatworms.[1][2]

They are found in both freshwater and marine environments, and also include a number of species found in damp terrestrial conditions. Most are free-living, but the group includes the genus Bdelloura, which lives comensally on the gills of horseshoe crabs. Seriatans are distinguished from other related groups by the presence of a folded pharynx and of a number of diverticula arising from the intestine. The intestine itself may be either simple or branched.[3]

With the Nematoda it looks very different. In the Nematoda its much worse. Most Nematoda are free-living, but we found only 2.63% of them. Blaxter et al. speaks of at least seven independently arosed parasitism [21]. In a recent article Blaxter identifies 18 origins [22] in Nematoda.

The problem at this point, however, is obvious: 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.

The evolution of parasitism in Nematoda:

"while only approximately 23 000 species have been described (J. Hallan, unpublished; https://insects.tamu.edu/research/collection/hallan/), the true species-level diversity may be 1 million or more (Lambshead, 1993)."

Phylum	# nodes	original states FL	original states P	final states 0 (FL)	final states 0.4	final states 0.5	final states 0.67	final states 0.75	final states 1 (P)
Chordata	91785	10451 99.83%	18 0.49%	91734 99.94%	0	0	0	0	51 0.06%
Nematoda	30127	21 0.63%	3289 99.37%	791 2.63%	0	1017 3.38%	0	0	28319 94%
Platyhelminthes	22683	7 0.1%	7086 99.9%	949 4.18%	0	151 0.67%	0	0	21583 95.15%
Apicomplexa	1863	1 0.39%	255 99.61%	1 0.05%	0	0	0	0	1862 99.95%
Arthropoda	1198981	18912 62.93%	11141 37.07%	1099509 91.7%	1313 0.11%	22478 1.87%	4176 0.35%	1665 0.14%	70223 5.86%

Table 3.5: Phylum (leaf nodes)

Kingdom	# nodes	original states FL	original states P	final states 0 (FL)	final states 0.25	final states 0.33	final states 0.4	final states 0.5	final states 0.67	final states 0.75	final states 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 3.6: Kingdom (inkl internal nodes)

" Estimates of the number of species of parasitic nematode per host suggest that there may be of the order of 25 000 nematode parasites just of vertebrates, most of which remain undescribed (Dobson et al. 2008)"
"a large proportion of nematode species may be parasites." [22]

Anmerkung: Das sind schonmal vier große Kontraste, wenn dann noch Zeit bleibt, die schwirigen... Arthropoden, Fungi, Pflanzen... (Emanuel)

Phylum	# nodes	original states FL	original states P	final states 0 (FL)	final states 0.4	final states 0.5	final states 0.67	final states 0.75	final states 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
Arthropoda	1319460	18912	11141	1207204	1313	25499	4852	1957	78635

Table 3.7: Phylum (inkl internal nodes)

Kingdom	# nodes	original states FL	original states P	final states 0 (FL)	final states 0.25	final states 0.33	final states 0.4	final states 0.5	final states 0.67	final states 0.75	final states 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 3.8: Kingdom (leaf nodes)

3.4.2 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

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

Table 3.9: Origins and losses

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 [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.

```
# possible tags: 0, 0.333, 0.4, 0.5, 0.667, 0.75, 1
# rounded to:    0  0      0    0    1      1     1
if node_state != father_state:
  if father_state == 0:
      origins += 1          # FL -> P
      new_found = True
  else:
      losses += 1           # P -> FL
      new_found = True
```

TODO: without rounding change else: to elif $father_state == 1$

3.4.3 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 ~ taxa	XXXXX	XXXXX	XXXXX	XXXXX
correct predicted ~ taxa + depth	117703	XXXXX	XXXXX	XXXXX
correct predicted ~ taxa * depth	117592	XXXXX	XXXXX	XXXXX

Table 3.10: Residuals of cross validation prediction

Resiudals:

- correct predicted ~ 1: 120325
- correct predicted ~ kingdom: 117877

Model / Taxa	Kingdom	Phylum	Class	Order
correct predicted $\sim$ taxa	117936	112242	XXXXX	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

		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

- correct predicted $\sim$ phylum: 111466

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:

		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

3.5 Effects of Taxa in the different models

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

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

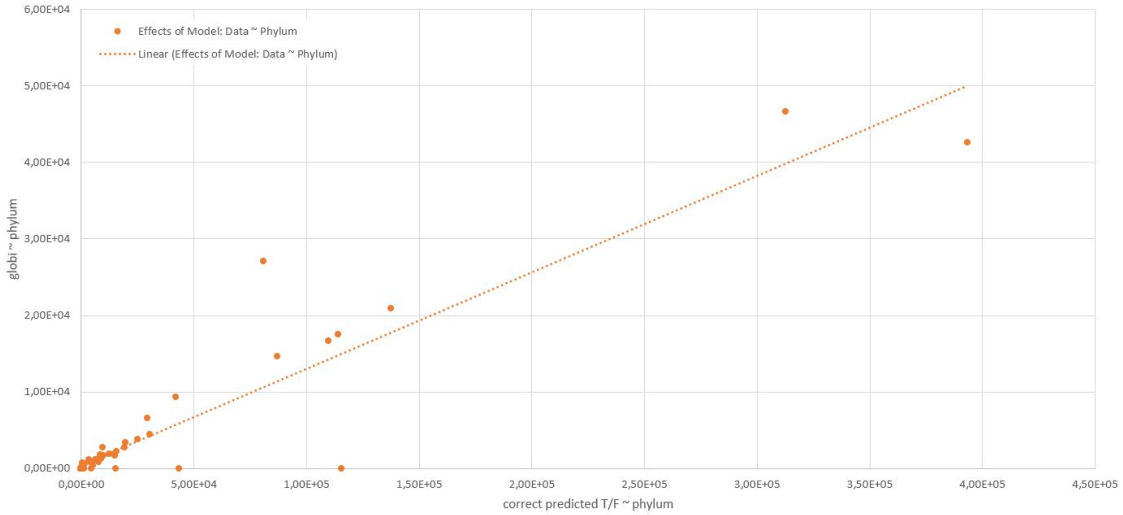


Figure 3.3: Effects of Model: Data $\sim$ Phylum

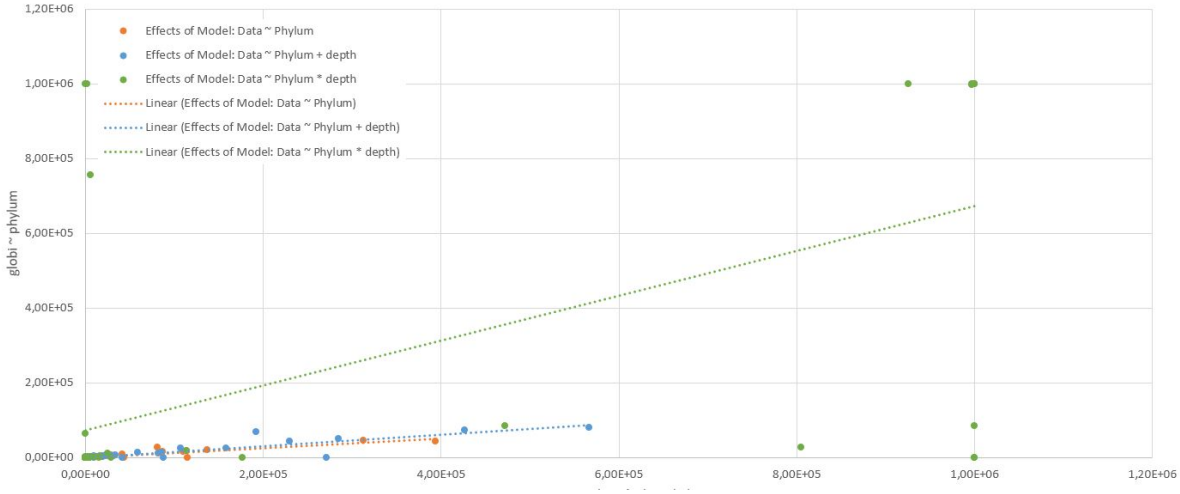


Figure 3.4: Effects of 3 Models: Data $\sim$ Phylum ($+$/* depth)

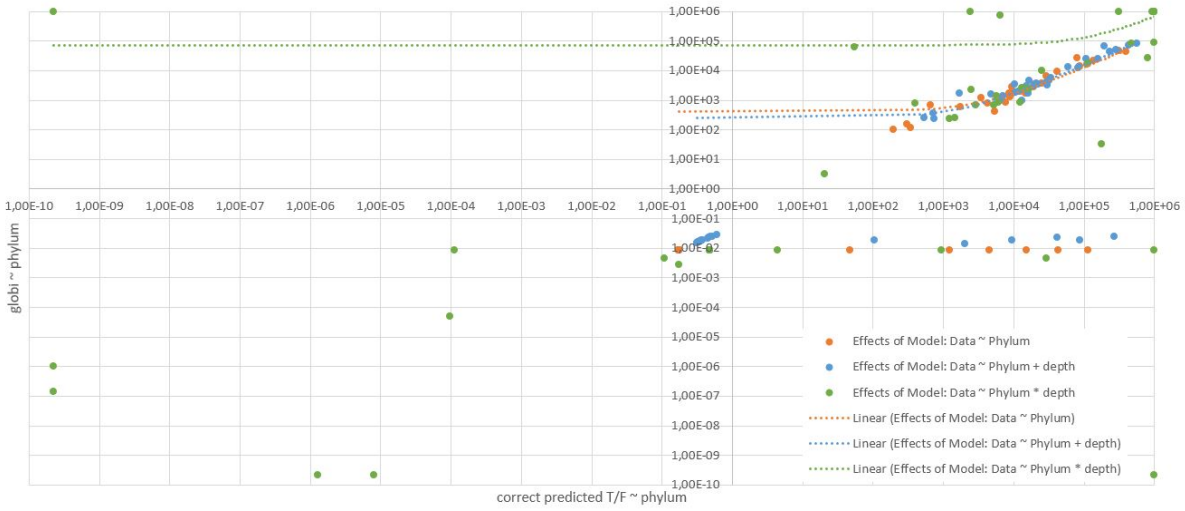


Figure 3.5: Effects of 3 Models: Data $\sim$ Phylum (+/* depth)
both axis in log scale

4 Discussion

Wie gut ist der randomisiert erstellte Baum?
Wie gut kommt unsere Simulation an die echte Datenlage heran.
Fehlerqoute der Daten an sich?
Wie gut ist unsere Datenlage? 3 mio knoten, 1.8 named species (leaf nodes), 200.000 leaf nodes mit Information.
Simulation von subtrees
Welche Teile des Baumes sind gut, an welchen muss noch viel geforscht werden.
Wieviele Origins haben wir gefunden, was bedeutet diese Zahl?

Parameter der Simulation:

- Wie ist die Verteilung der vergessenen internen Knoten? Zum Wurzelknoten hin mehr vergessen?
- Wie sehen die übergangswahrscheinlichkeiten aus von P→FL und andersherum?
- Verteilung Parasiten zu Freilebend zu keine Information

Selecting of the 'right' / best Distribution

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5 Appendices

5.1 OTL analysis

5.1.1 List of all phyla

Phyla (53):
Acanthocephala, Amoebozoa, Apicomplexa, Arthropoda, Ascomycota, Bacillariophyta, Basidiomycota, Brachiopoda, Bryozoa, Chaetognatha, Chlorophyta, Chordata, Chromerida, Chytridiomycota, Ciliophora, Cnidaria, Colponemidia, Ctenophora, Cycliophora, 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

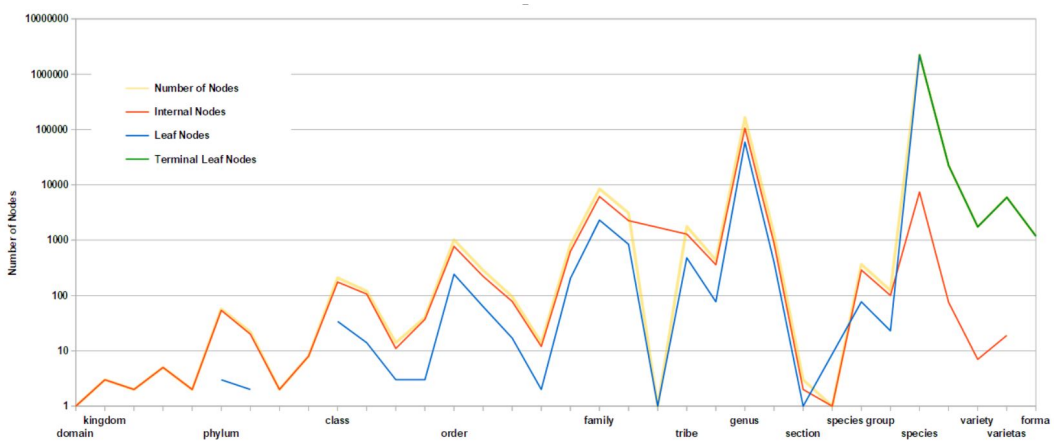

Figure 5.1: Distribution of Nodes in Rank-Cathegories

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 5.1.
... (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).

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: ...

6 Komplett aussortierte Tabellen etc

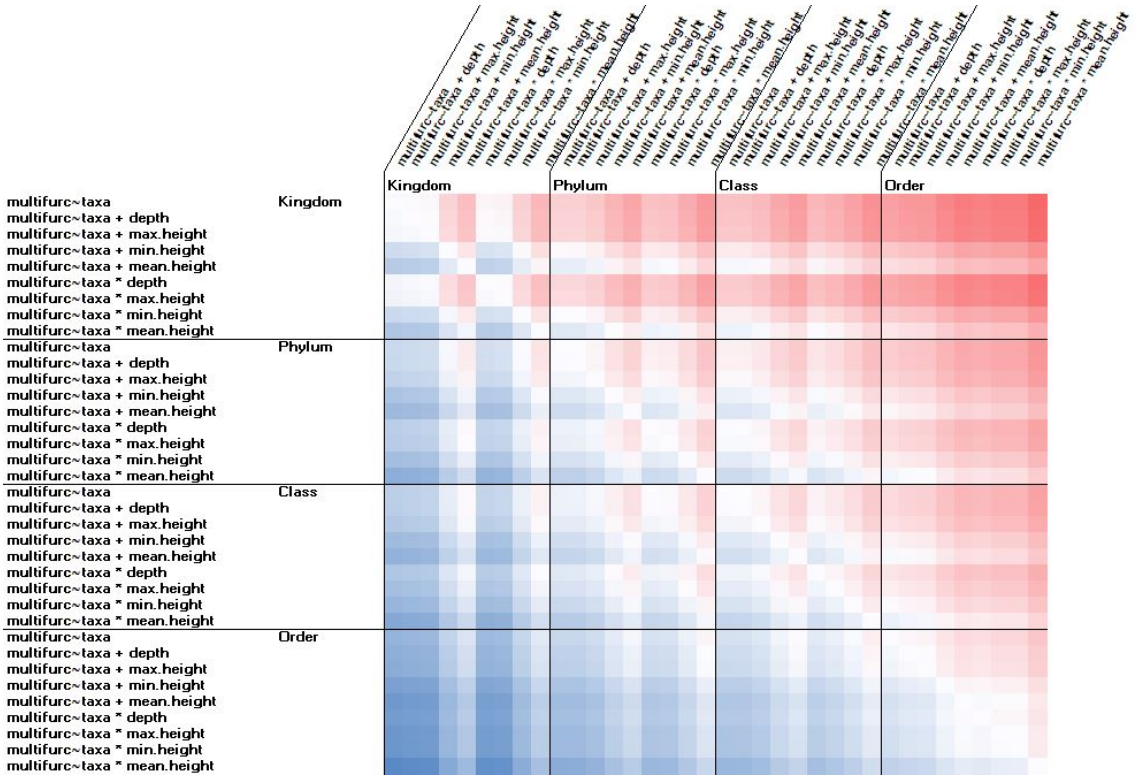

Figure 6.1: Crosstable of Residual values

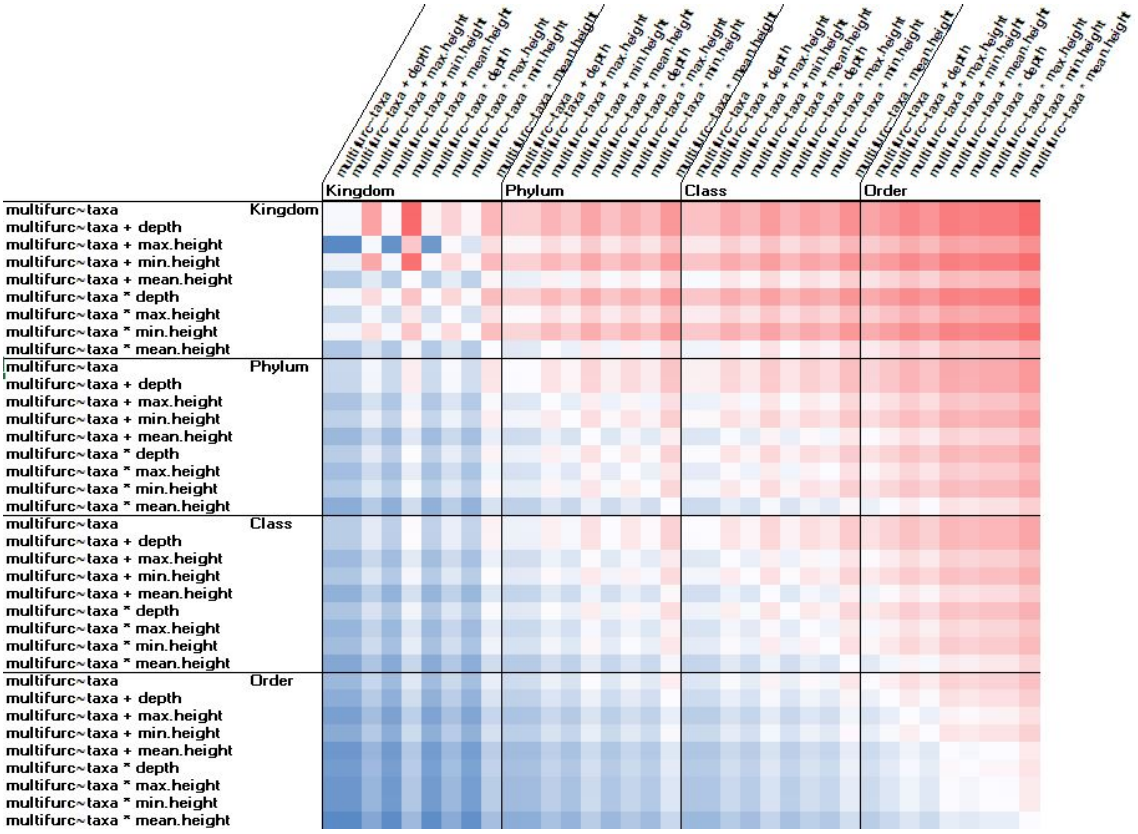

Figure 6.2: Crosstable of BIC values