Biology Department
Research Group: Systematic & Evolutionary Botany

GENERATION TIME, A DRIVING FACTOR OF SUBSTITUTION RATE HETEROGENEITY IN THE PANTROPICAL PLANT FAMILY ANNONACEAE

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Introduction

Evolution is the process by which properties of populations of organisms change over time. This process is also known as descent with modification (Darwin, 1909). Evolution depends on the passage of genetic information from one generation to the next. In order for genetic information to be inherited, DNA must be replicated. During replication, errors or so-called mutations can occur. A mutation generates a new allele, which is a variant of a gene, in a single individual and can be inherited by the descendants and increase in frequency over time until the mutant allele replaces all other alleles and becomes fixed. These mutations are the ultimate source of genetic variation, without them there would be no variation and no evolution. When a mutation in a single individual becomes fixed over time and is now carried by all members of the population it is called substitution and this forms the basic process of molecular evolution. Molecular evolution is the process of evolutionary change at the level of DNA sequences.

The frequency with which an allele occurs in a population is determined by two main processes: natural selection and genetic drift (Darwin, 1909; Masel, 2011). When a mutated allele increases an individual's survival and number of produced offspring natural selection will increase the frequency of this 'fitness-increasing' mutation until it becomes fixed in the population. On the other hand, if a mutation reduces the fitness of an individual natural selection will reduce the frequency of this mutation until it is lost in the population. Besides natural selection, genetic drift also drives the fate of alleles (Masel, 2011). The frequency of alleles can fluctuate due to random events and an allele can become lost or fixed by chance. However, most mutations are neutral and do not have an advantage or disadvantage, hence these neutral alleles are not subject to natural selection and their fate is driven by genetic drift. In 1968 it was proposed that most of the evolution of DNA sequences occurs by chance rather than by natural selection (Kimura, 1968; Kimura, 1983). The neutral theory allows us to explore patterns of evolution and predicts the rate of neutral substitution because it depends on a single parameter, the mutation rate. It was shown that the rate of substitution of neutral mutations is equal to the mutation rate and thus the more neutral mutations occur the more substitutions there will be (Kimura, 1968; Yi, 2013). As a consequence, if the mutation rates are constant over time then the substitution rates should also be constant.

In the early studies of molecular evolution it was postulated that DNA and protein sequences evolve at a constant rate over time. This concept of a constant substitution rate is called the molecular clock hypothesis (Zuckerkandl and Pauling, 1965). However, over the years evidence against the molecular clock hypothesis has increased enormously (Wu and Li, 1985; Lemey et al., 2009) and it has been demonstrated that there is significant variation in rates of substitution at different levels. Earlier studies (Britten, 1986) have observed that the rate of DNA change differs between phylogenetic groups, where the slowest rates were found in birds and primates and the fastest rates were found in mice and Drosophila. Rate heterogeneity has not only been observed among evolutionary lineages, but also among genomes and genes (Drouin et al., 2008; Zhu et al., 2014). The mitochondrial, chloroplast and nuclear genomes and genes also differ from each other in rates of substitution (Drouin et al., 2008; Zhu et al., 2014). At last variation in substitution rates was also demonstrated among individual codon positions of DNA sequences (Simmons et al., 2006).

Several factors such as environmental energy (Davies et al., 2004), population size (Woolfit and Bromham, 2005) and life-history traits (Bromham et al., 1996) have been proposed to be the drivers of the variation seen in substitution rates. Life-history traits include body size, longevity, fecundity, generation time etc. and they all seem to affect the rate of molecular evolution. Smaller species, which produce a high number of offspring and have a short lifespan, have faster rates of substitution than larger species. However, many life-history traits covary with each other and this correlation between the body size and rate of substitution is interpreted as an indirect effect of the covariation with other life-history traits, such as generation time and metabolic rate, which are thought to influence the rate of substitution. It is generally accepted that a species with a small body size, rapid generation time, high fecundity and short lifespan tends to have faster rates of substitution (Bromham, 2011).

Relationships between species can be reconstructed with the use of phylogenetic trees. The branch length of each branch on a phylogenetic tree is governed by the number of substitutions that occurred and the amount of time a lineage had to accumulate those substitutions. Substitution models, such as the molecular clock model, can be used to estimate the rate of substitution over time. If we can infer the substitution rate, we can estimate the divergence time between two species. Conversely, if the divergence time is known, we can estimate the rate of substitution. Evidence against the molecular clock model has been increasing over the years, demonstrating that there is rate heterogeneity (Britten, 1986; Simmons et al., 2006; Drouin et al., 2008; Lemey et al., 2009; Zhu et al., 2014). Consequently, different models, that relax the assumptions of the molecular clock model, have been developed to accommodate this rate heterogeneity (Sanderson, 1997; Thorne et al., 1998). There are two main groups of relaxed clock models: autocorrelated relaxed clock model and uncorrelated lognormal clock model. According to the autocorrelated relaxed clock model the rates of substitution in daughter lineages are inherited from the parental branches, resulting in a gradual rate change between neighbouring lineages (Dornburg et al., 2012; Fourment & Darling, 2018). An alternative model is the uncorrelated relaxed clock model, which allows each branch of a phylogenetic tree to have its own rate. Thus, in that case, the rates of substitution are independent across successive branches (Lartillot et al., 2016).

In this study we will explore the correlation between generation time and substitution rates among lineages in plants and test if differences in generation time between species provide a good explanation for the observed variation in substitution rates. Generation time can be defined as the average amount of time an individual of a certain species takes to reproduce for the first time (Page and Holmes, 2009; Bromham, 2016). Previous studies (Li et al., 1987; Gaut et al., 1996) proposed the generation time hypothesis, which states that a species with a shorter generation time copies its genome more often per time unit, resulting in more germline-cell divisions, thereby having more opportunities to accumulate DNA replication errors compared to a species with a longer generation time. So far, the generation time hypothesis has been demonstrated in mammals (Li et al., 1987; Ohta, 1993; Bromham et al., 1996), birds (Mooers and Harvey, 1994), invertebrates (Thomas et al., 2010; Saclier et al., 2018), between annuals and perennials (Laroch and Bousquet, 1999; Andreasen and Baldwin, 2001) and between woody plants and herbs (Kay et al., 2006; Smith and Donoghue, 2008). We could say that generation time is a good proxy for the overall germline-cell replication (Lanfear et al., 2013). However, plants lack a germline, they grow from an apical meristem through continuous mitosis and only late in plant development gametes are formed (Soria et al., 2008; Gaut et al., 2011). Thus, the correlation between generation time and substitution rate is less straightforward compared to animals and this could explain why the correlation with generation time is absent in some studies (Whittle and Johnston, 2003). But because gametogenesis occurs late in plants, somatic mutations acquired during vegetative growth can be transmitted to the next generation (Watson et al., 2016). Therefore, the generation time hypothesis can be modified for plants and it is very likely that fast-evolving plants such as annuals and herbs, that have a short time to flowering, have shorter generation times and experience higher rates of cell division, resulting in more opportunities to accumulate DNA replication errors compared to slow-evolving plants, for example perennials and trees (Lanfear et al., 2013).

This study will be the first that aims to test the correlation between generation time and substitution rate among species of different clades of the pantropical plant family Annonaceae, the most species-rich family belonging to the order of the Magnoliales. The Annonaceae are a large family of flowering plants, mainly trees and lianas, that consist of ca. 2500 species occurring predominantly in tropical forests. The phylogeny of the Annonaceae has already been reconstructed and currently four main clades are recognized: the Annonoideae, the Malmeoideae and two species-poor clades (Anaxagoroideae and Ambavioideae) that form the basal lineages (Chatrou et al., 2012). The four subfamilies of the Annonaceae were distinguished based on several morphological characteristics such as leaf arrangement, shape of seed, habit etc. (Maas and Westra, 1985; Maas et al., 2003; Couvreur, 2009). The Annonoideae are characterised by a woody climber habit and free inner petals for example, while the Malmemoideae are characterised by a tree habit and connate inner petals (Lestari et al., 2017). In early papers (e.g. Richardson et al., 2004) it was noted that the Annonoideae and Malmeoideae differ significantly in branch lengths, which is why they were called the long branch clade and the short branch clade, respectively. Hoekstra et al. (2017) demonstrated that the differences in branch length reflect differences in substitution rates. However, these previous studies used limited datasets with only a few number of species and this could be a crucial factor influencing the results and conclusions that were made. Now, we will use a large dataset with many different species and try to assess whether generation time is a factor that drives and explains the variation seen (Hoekstra et al., 2017) in substitution rates between the two large subfamilies, the Annonoideae and Malmeoideae, of the flowering plant family Annonaceae. With this study we try to contribute to the understanding of the complex evolutionary relationships among the Annonaceae and to unravel the evolutionary processes that have formed the variation in substitution rates among the Annonaceae.

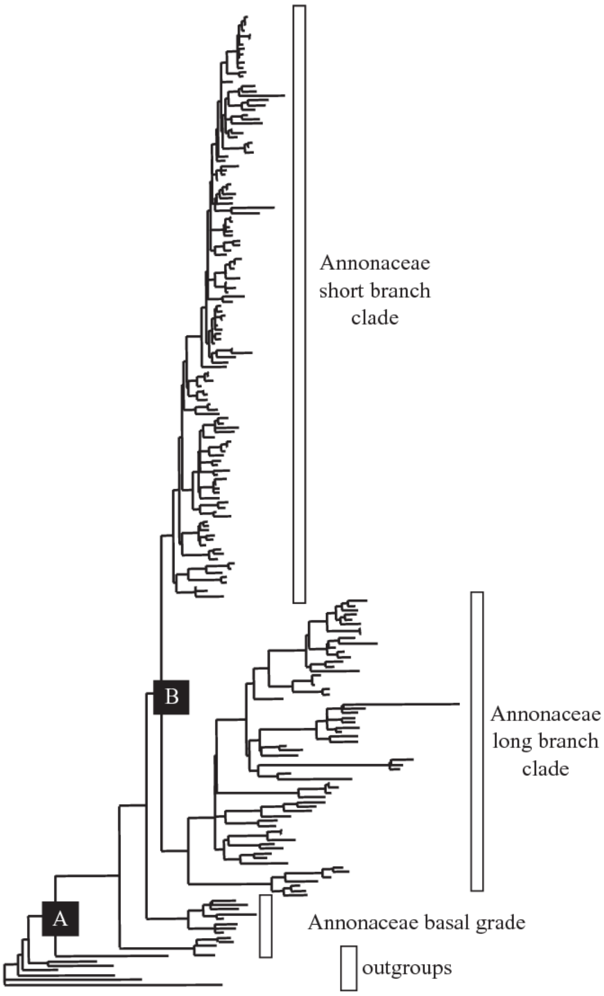

Figure 1. Phylogenetic tree of the Annonaceae showing the two large subfamilies Annonoideae and Malmeoideae, called respectively the long branch clade and the short branch clade. *Note*. Taken from Richardson et al. (2004)

Objectives

Earlier studies (Richardson et al., 2004; Hoekstra et al., 2017) have demonstrated that the Annonoideae (long branch clade) and Malmeoideae (short branch clade), two subfamilies of the Annonaceae, differ significantly in terms of branch lengths and that these differences reflect variation in substitution rates. However, the taxon sampling in earlier studies (Hoekstra et al., 2017) was limited. Therefore, during this thesis project, we will try to assess whether these significant differences in substitution rate, for which we will use the branch length as proxy, between the Annonoideae and Malmeoideae are also present when using a large dataset with much more species compared to Hoekstra et al. (2017). Secondly, we will test whether generation time, for which we use the mortality rate as proxy, differs between the Annonoideae and Malmeoideae. Finally, we will try to determine whether generation time is a factor that drives the variation in substitution rate.

For the first objective we expect to observe significant differences in substitution rates between the Annonoideae and Malmeoideae, as this difference was already observed in previous studies (Hoekstra et al., 2017; Richardson et al., 2004). Since the Annonoideae are called the long branch clade and we use the branch length as proxy for the substitution rate, we expect to observe higher substitution rates for the Annonoideae. Baker et al. (2014) as well used the mortality rate as a measure for the generation time and this study already mentioned differences in mortality rate between genera. Therefore, for the second objective, we expect significant differences in generation time/mortality rate between the two major clades. Finally, the generation time hypothesis which states that the shorter the generation time the more errors can be copied to the next generation and thus the higher the substitution rate, has already been demonstrated in mammals (Li et al., 1987; Bromham et al., 1996), birds (Mooers and Harvey, 1994), invertebrates (Thomas et al., 2010) and also in plants (Smith and Donoghue, 2008; Laroch and Bousquet, 1999). Because of this previous evidence we would expect to observe a clear negative correlation between generation time and substitution rate, indicating that generation time is a factor that drives the variation in substitution rate.

Method

Molecular data acquirement and sequence alignments

We assembled molecular sequence data for 819 species of the flowering plant family Annonaceae (**Appendix: part A, table 1**). Five different chloroplast markers were used: *matK*, *ndhF*, *psbA-trnH*, *rbcL* and *trnLF*. For *matk* 603 species were sequenced, for *ndhF* 398 species, for *psbA-trnH* 580 species, for *rbcL* 683 species and for *trnLF* 730 species were sequenced. The molecular data were obtained from GenBank. The dataset included species from all subfamilies of the Annonaceae, sampling ten species of Ambavioideae, five species of Anaxagoroideae, 530 species of Annonoideae and 268 species of Malmeoideae. Six species were sampled as outgroups, being part of the following families: Degeneriaceae (*Degeneria vitiensis* I.W. Bailey & A.C. Sm.), Eupomatiaceae (*Eupomatia bennettii* F. Muell.), Himantandraceae (*Galbulimima belgraveana* Sprague), Lauraceae (*Persea americana* L.), and Magnoliaceae (*Liriodendron chinense* (Hemsl.) Sarg. and *Magnolia Kobus* DC.). To infer the evolutionary relationships among lineages we must distinguish homologies, characteristics that are shared between species due to common ancestry, from homoplasy, similarities that are not the result of shared common ancestry. The process of sequence alignment is very important in DNA sequence analyses as it establishes homologous sites which allows us to reconstruct phylogenetic trees, displaying the similarities between related lineages, and to detect evolutionary changes over time. Each used marker was aligned using MAFFT v.7450 (Katoh et al., 2002; Katoh and Standley, 2013) by the means of the software program Geneious Prime version 1.1.2021 (https://www.geneious.com). All alignments were concatenated, comprising all the 819 species and 6280 positions.

Phylogenetic analyses

We used PartitionFinder2 (Lanfear et al., 2016) on the CIPRES Gateway server (https://www.phylo.org) to determine the best partition scheme and molecular models for all markers. The best partition schemes were used in all further phylogenetic analyses. The phylogenetic tree of Annonaceae was first inferred using the combined matrix and the Bayesian criterium by using MrBayes version 3.2 (Ronquist and Huelsenbeck, 2003) on CIPRES Gateway. First we converted the dataset to a NEXUS file. The evolutionary models recovered with PartionFinder2 were all the general time-reversible (GTR) nucleotide substitution model (nst = 6 in MrBayes) with gamma distributed rates and a proportion of invariable sites. Each analysis ran for ca. 50 000 000 generations with 2 parallel runs, each with four independent Metropolis-Coupled Markov Chain Monte Carlo chains. We assessed convergence and stationarity using the R package coda (Plummer et al., 2006) by visually observing the plots of parameter values and log-likelihood against the number of generations. We assumed that there was chain convergence when the average standard deviation of split frequencies was circa 0.01 or less. As burn-in, we discarded 20% of the trees. The mcc tree was searched using the R package phangorn (Schliep, 2011).

The inferred phylogenetic tree was calibrated using a Bayesian approach to accurately determine the divergence times and evolutionary rates. We used the program BEAUTi to set the model parameters for the analysis in BEAST version 1.10 (Suchard et al., 2018) available in CIPRES Gateway server. We fixed the tree topology using the phylogram previously obtained and only sampled trees with different branch lengths. To do this we switched off all operators changing the topology of the tree. The ages of the stem and crown nodes of Annonaceae were fixed using fossil and previous dating information (Thomas et al., 2015; Couvreur et al., 2011; Pirie and Doyle, 2012). To calibrate the phylogeny, two fossils, *Endressinia brasiliana* and *Futabanthus asamigawaensis*, were used. *Endressinia brasiliana* is a fossilized flowering shoot from the Aptian (ca. 112 Ma ago) of Brazil (Mohr and Bernardes de Oliveira, 2004). *Endressinia brasiliana* provides a minimum age for the Magnoliineae clade, which consist of *Degeneria*, *Galbulimima*, *Eupomatia* and Annonaceae (Doyle and Endress, 2010), thereby fixing the root node to 112 Ma. The second fossil, *Futabanthus asamigawaensis*, is a fossilized flower found in Japan from the early Coniacian (ca. 89 Ma ago) and is considered as the earliest probable fossil of the Annonaceae (Takahashi et al., 2008). An uncorrelated relaxed clock model was implemented, using the Yule process. For both the root and crown taxon groups we have set an uniform prior distribution and we limited the age for the root taxon group from 112 to 156 Ma and for the crown taxon group from 89 to 112 Ma. Two independent runs ran for ca. 50 000 000 generations. The posterior files were edited using the R package coda (Plummer et al., 2006). As burn-in, we discarded 20% of the initial samples. The mcc tree was searched using the R package phangorn (Schliep, 2011).

Statistical analyses

To evaluate the putative correlation between substitution rates and generation time in Annonaceae, we used as proxies respectively the branch lengths of phylogenetic trees and the mortality rates. The branch lengths were recovered from the phylogram, obtained with MrBayes, and are measured in substitutions per site (subs./site). We also used the substitutions per site per million years (subs/site/my). The latter metric was estimated using both the phylogram and the chronogram, obtained with BEAST. We divided the branch lengths of the phylogram by the branch lengths of the chronogram, recovering the branch values in substitutions per site per my (**Fig. 2**). Since the topologies are identical, all branches of both phylogram and chronogram were used. Branch length transformations and edition were carried out in R using the packages APE version 4.0.5 (Paradis et al., 2004), and phangorn version 4.0.5 (Schliep, 2011). The mortality rate data made available for tropical tree families were used to recover the mortality rates for Annonaceae species (Baker et al., 2014). The mortality rate represents the percentage of trees, with > 10 cm diameter, that die per year on average for each species. The mortality rate was obtained for 31 species of the Annonaceae that also were included in our phylogenetic tree (subs/site/my) (**Appendix: part C, table 2**). Generation time is calculated as 1/mortality and therefore we will use the mortality rate as a measure for generation time.

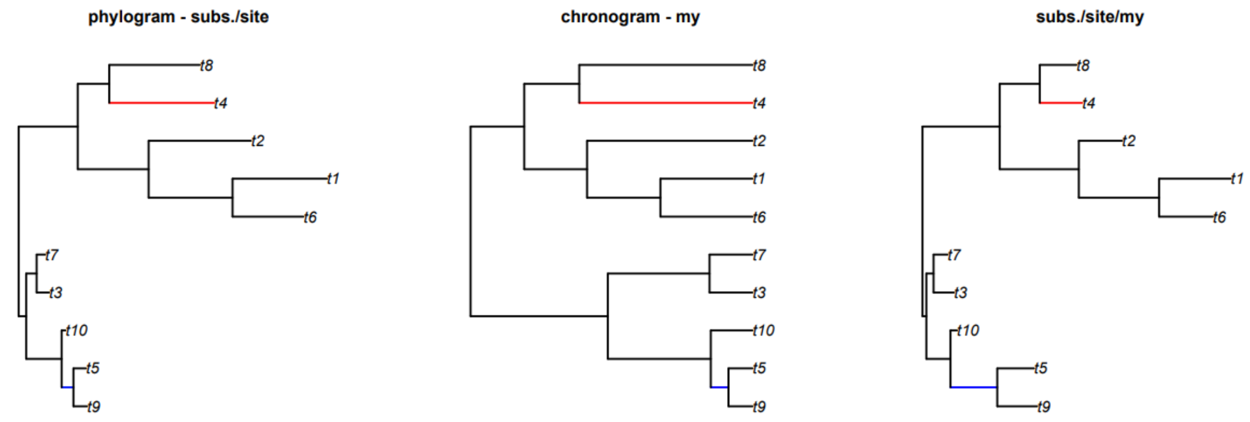

Figure 2. Left: The phylogram with branch lengths expressed as substitutions/site. Middle: The chronogram with branch lengths expressed as million years (Ma). Right: The tree, formed by dividing the branch lengths of the phylogram by the branch lengths of the chronogram, with branch lengths expressed as substitutions/site/million years.

To test the effect of mortality rates on branch lengths we compared the subfamilies Annonoideae, and Malmeoideae. Differences in branch lengths between the two subfamilies were previously reported (Richardson et al., 2004; Couvreur et al., 2019) and formally tested using genomic data (Hoekstra et al., 2017). As our first step, we evaluated the differences in branch lengths between subfamilies using the species-level phylogeny of Annonaceae made available here. We tested whether the branch lengths of the Malmeoideae were significantly smaller than the branch lengths of the Annonoideae using the whole clades and the Wilcoxon rank sum test in R (**Appendix: part B**). To correct for the number of species sampled for each subfamily, we also reduced the sampling of Annonoideae to 268 species, randomly resampling the tips ten times. With this strategy, we reduced the effect of the number of nodes in the branch lengths of the most diverse clade. To implement the comparison, the branch lengths were measured using both substitutions/site, and substitutions/site/my. A p-value equal or lower than 0.05 was considered significant. The statistical tests were carried out using R functions from the packages APE version 4.0.5 (Paradis et al., 2004), and phangorn version 4.0.5 (Schliep, 2011). Tree plots were generated using phytools version 4.0.5 (Revell, 2012) and boxplots using ggplot2 (Wickham, 2016) (**Appendix: part B**).

To estimate the correlations, we associated branch length values to terminals using two different measures. First, we used the path length, summing the branch lengths from the crown node of the subfamily to each individual terminal (**Fig. 3**) using the R package adephylo version .4.0.5 (Jombart and Dray, 2010). We also used the terminal branch lengths for all species (**Fig. 3**). To test if the differences in substitution rates between subfamilies are also recovered using the two measures, we used all the species sampled for all subfamilies. The steps described above to compare the subfamilies were used.

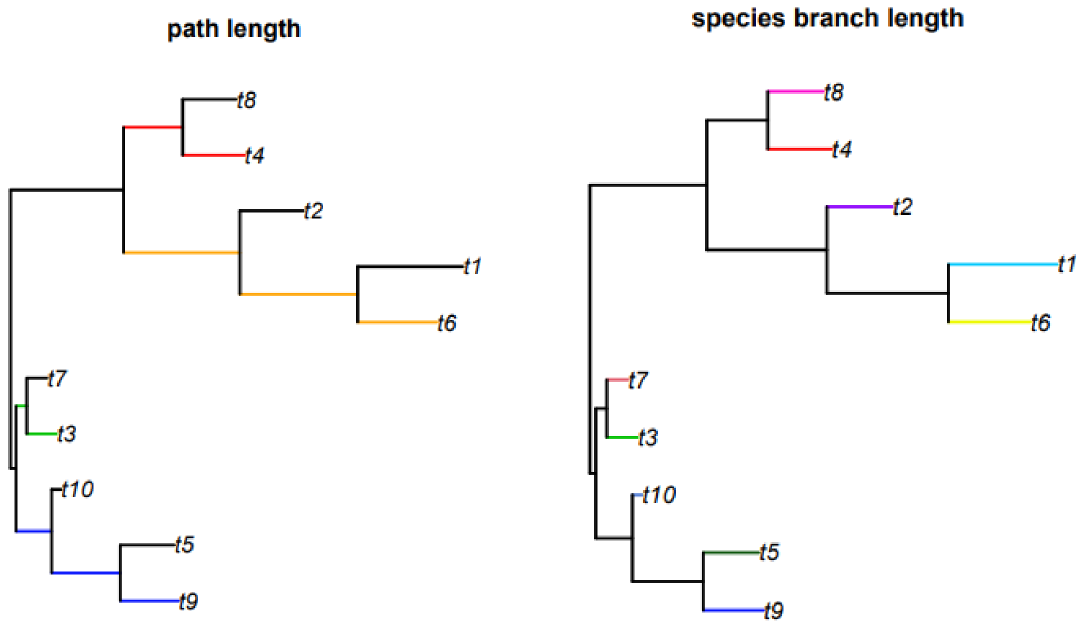

Figure 3. Left: The path length which is the sum of the branch lengths from the crown node of the subfamily to each terminal. Right: The species branch length which is the terminal branch length for each species.

We tested whether the generation time of the Annonoideae were significantly smaller than the generation time of the Malmeoideae using the Wilcoxon rank sum test in R. The correlations were tested using both Generalized Least Squares (gls; Kmenta, 1986), and Phylogenetic Generalized Least Squares (pgls; Martins and Hansen, 1997). Both gls and pgls are linear regressions evaluating the correlation between the residuals. The idea behind the pgls method is to correct the effect of the phylogeny on the data, reducing the variance. The correlations were estimated contrasting the path branch lengths/species branch lengths and mortality rates/generation times. A p-value equal or lower than 0.05 was considered significant. The correlation tests were carried out using R functions from the package nlme (Pinheiro et al., 2021; **Appendix, part D**).

Results

Phylogeny

The phylogenetic analyses on the concatenated dataset retrieved a phylogeny of the Annonaceae, including the Malmeoideae (short branch clade) and Annonoideae (long branch clade) (**Fig. 4, 6**). The phylogram (substitutions/site) and the chronogram (million years) demonstrate the distinction between the short branch clade and the long branch clade (**Fig. 4, 6**) as we clearly see a difference in path branch lengths and species branch lengths between the two subfamilies (**Fig. 5**). The tree (substitutions/site/million years), generated by dividing the phylogram by the chronogram, also demonstrates the clear distinction between the short branch clade and the long branch (**Fig. 7**).

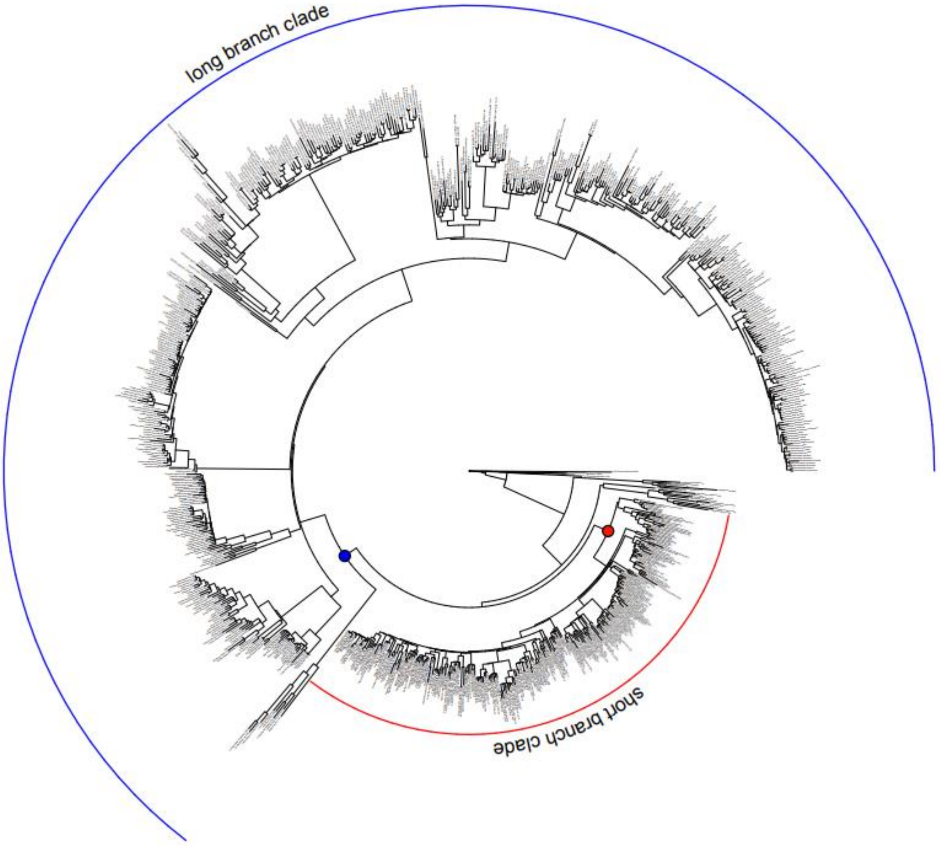

Figure 4. Phylogram of the Annonaceae (branch lengths expressed as substitutions/site). The blue line indicates the Annonoideae and the red line indicates the Malmeoideae. The blue dot represents the crown node of the Annonoideae and the red dot represents the crown node of the Malmeoideae.

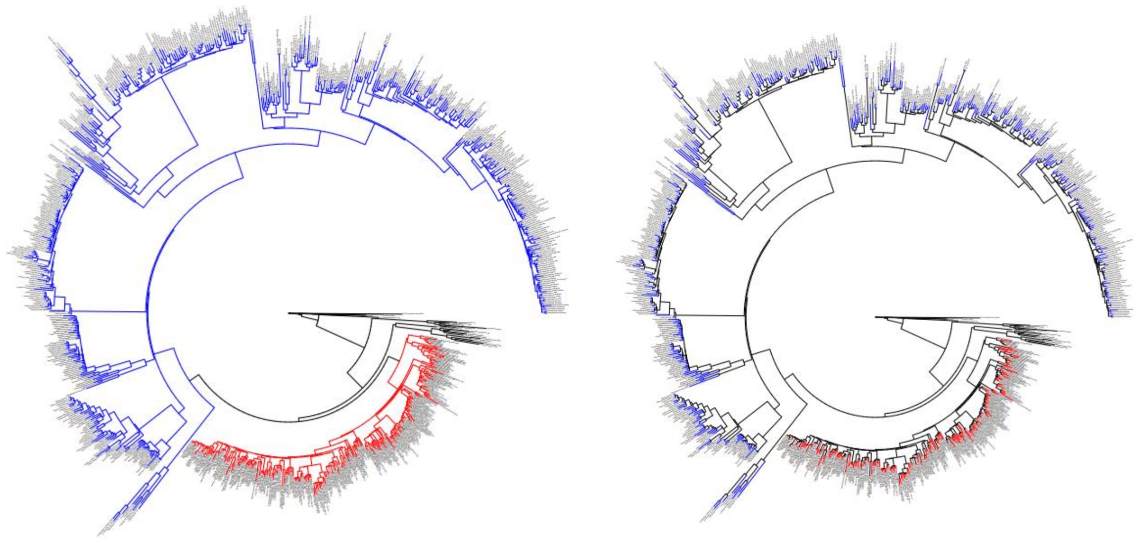

Figure 5. Phylogram (substitution/site) of the Annonaceae. Left: Blue lines represent the path branch lengths of the Annonoideae, while the red lines represent the path branch lengths of the Malmeoideae. Right: Blue lines represent the species branch lengths of the Annonoideae, while the red lines represent the species branch lengths of the Malmeoideae.

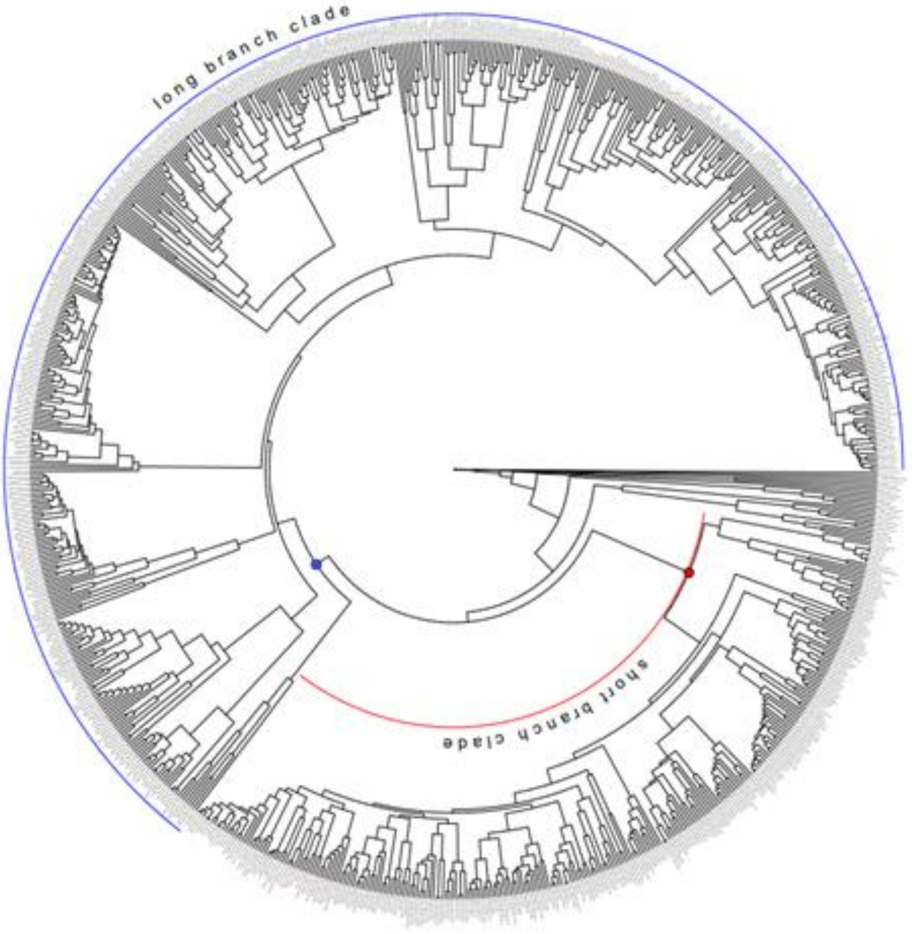

Figure 6. Chronogram (branch lengths expressed as million years) of the Annonaceae. The blue line indicates the Annonoideae and the red line indicates the Malmeoideae. The blue dot represents the crown node of the Annonoideae and the red dot represents the crown node of Malmeoideae.

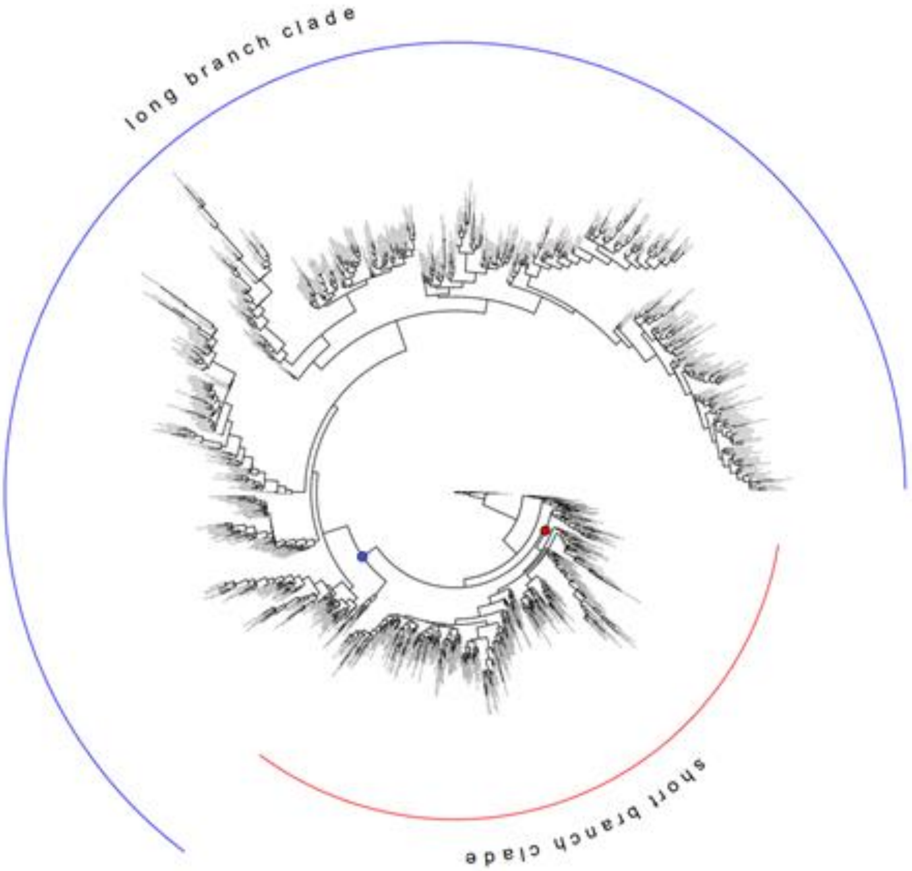

Figure 7. Tree of the Annonaceae (branch lengths expressed as substitutions/site/million years). The blue line indicates the Annonoideae and the red line indicates the Malmeoideae. The blue dot represents the crown node of the Annonoideae and the red dot represents the crown node of the Malmeoideae.

Statistical analyses: Evolutionary rates

When analysing the path branch lengths of the tree (substitutions/site/my) significant differences (p-value < 0.05) were found between the Malmeoideae and Annonoideae, confirming the hypothesis that the path branch lengths of the Malmeoideae are smaller than the path branch lengths of the Annonoideae (**Figs. 8, 9**). For the species branch lengths no significant differences (p-value > 0.05) were found, contradicting our hypothesis (**Figs. 10, 11**). However, for this part of the analyses the Annonoideae had more tips compared to the Malmeoideae, which could explain why no significant differences (p-value > 0.05) were found for the species branch lengths. After reducing the number of tips for the Annonoideae, significant differences (p-value < 0.05) were found for both the path branch lengths (**Figs. 12, 13**) and the species branch lengths (**Figs. 14, 15**) between the Malmeoideae and the Annonoideae. Thus we can already conclude that the substitution rate, which is proportional to the species branch length and path branch length, differs significantly between the Malmeoideae (short branch clade) and Annonoideae (long branch clade) and that the substitution rates are larger for the Annonoideae than for the Malmeoideae.

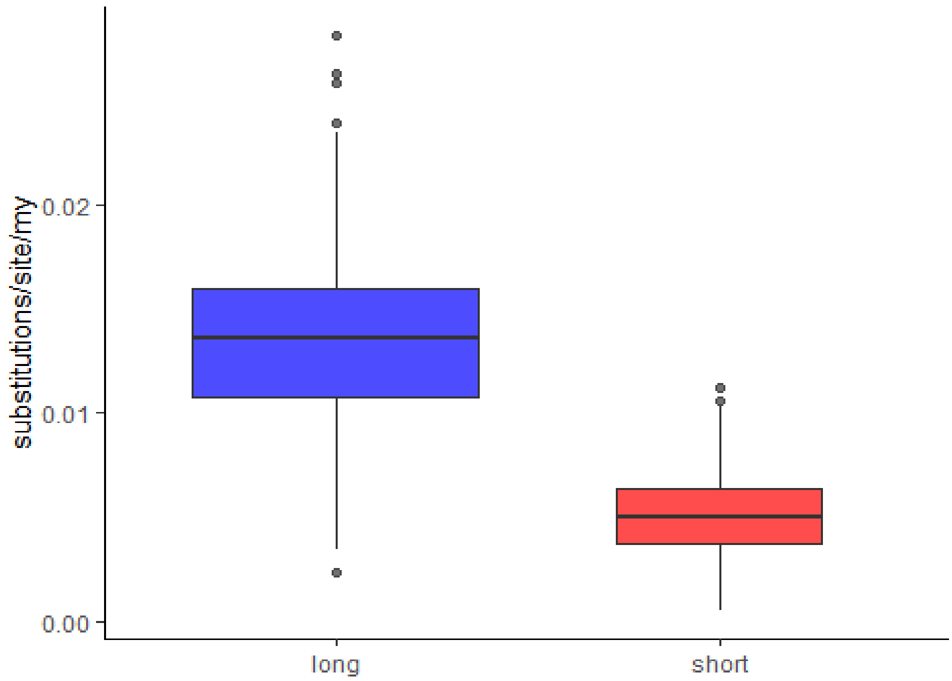

Figure 8. Boxplot showing difference in path branch length, which is the sum of the branch lengths from the crown node of the subfamily to each individual terminal. The path branch lengths of the Annonoideae are larger than the path branch lengths of the Malmeoideae.

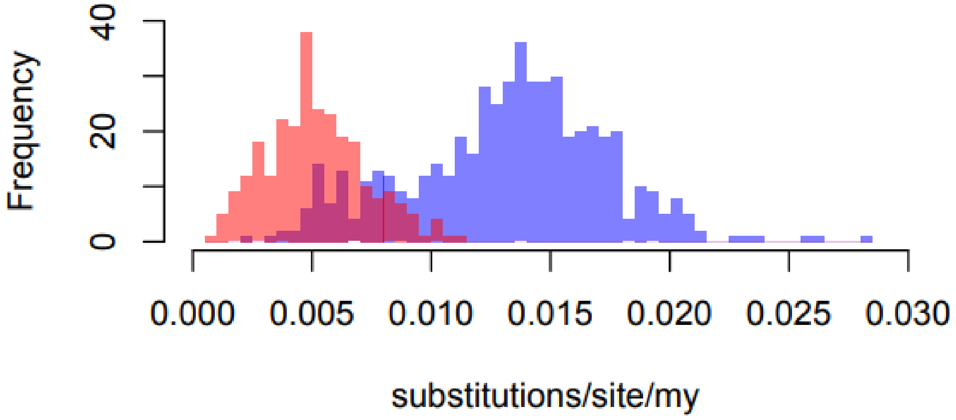

Figure 9. Histogram showing the difference in path branch length between the Malmeoideae (red) and the Annonoideae (blue). The dark red represents the overlap in path branch lengths between the Malmeoideae and the Annonoideae. The path branch lengths of the Annonoideae are larger than the path branch lengths of the Malmeoideae.

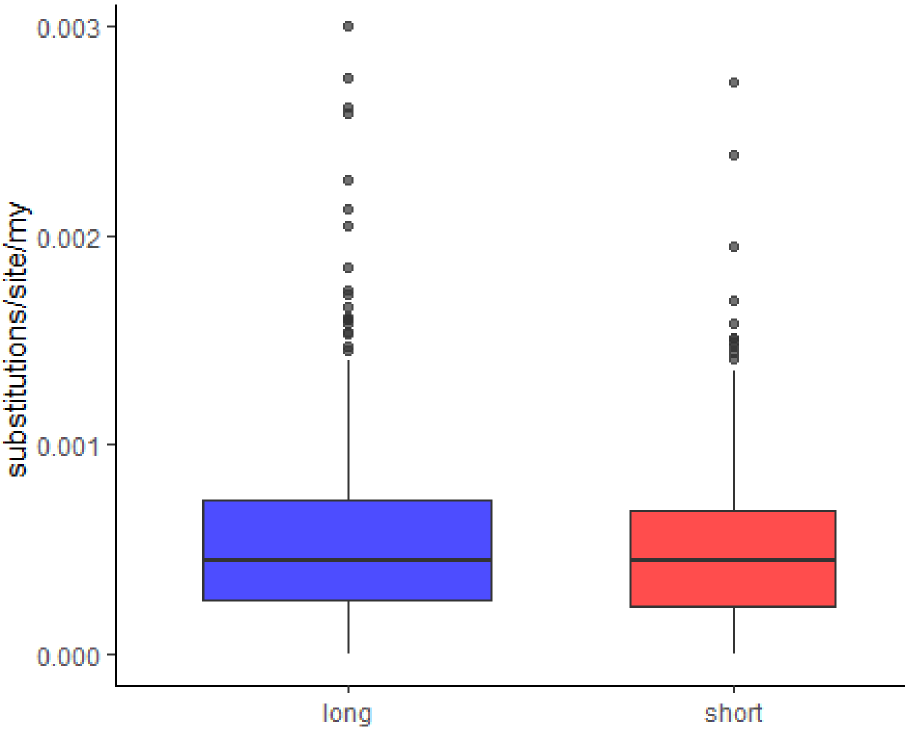

Figure 10. Boxplot showing difference in species branch length, which is the terminal branch length for each species. The species branch lengths do not differ between the Annonoideae and the Malmeoideae.

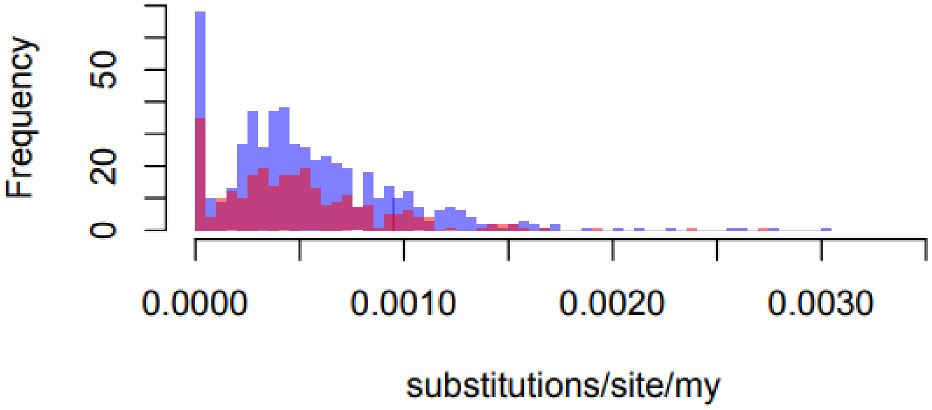

Figure 11. Histogram showing the difference in species branch length between the Malmeoideae (red) and the Annonoideae (blue). The dark red represents the overlap in path branch lengths between the Malmeoideae and the Annonoideae. The path branch lengths of the Annonoideae and the Malmeoideae are similar.

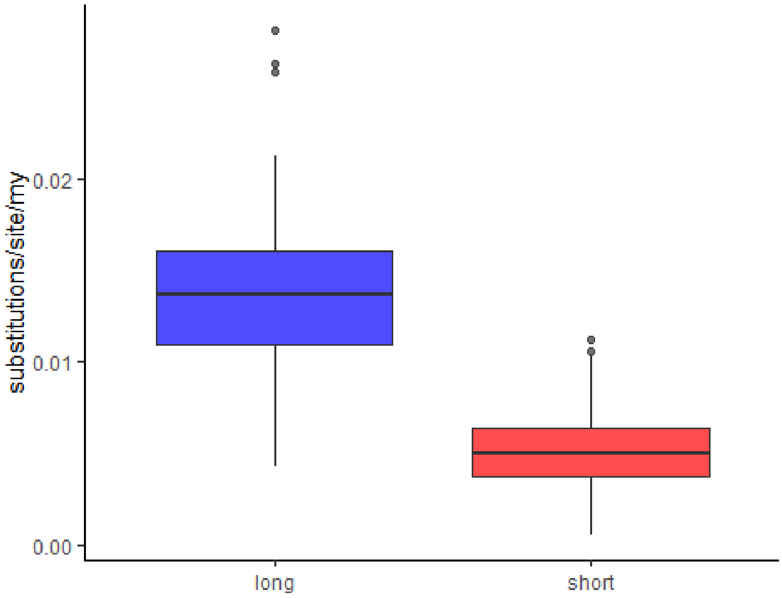

Figure 12. Boxplot showing difference in path branch length, the sum of the branch lengths from the crown node of the subfamily to each individual terminal. The path branch lengths of the Annonoideae are larger than the path branch lengths of the Malmeoideae.

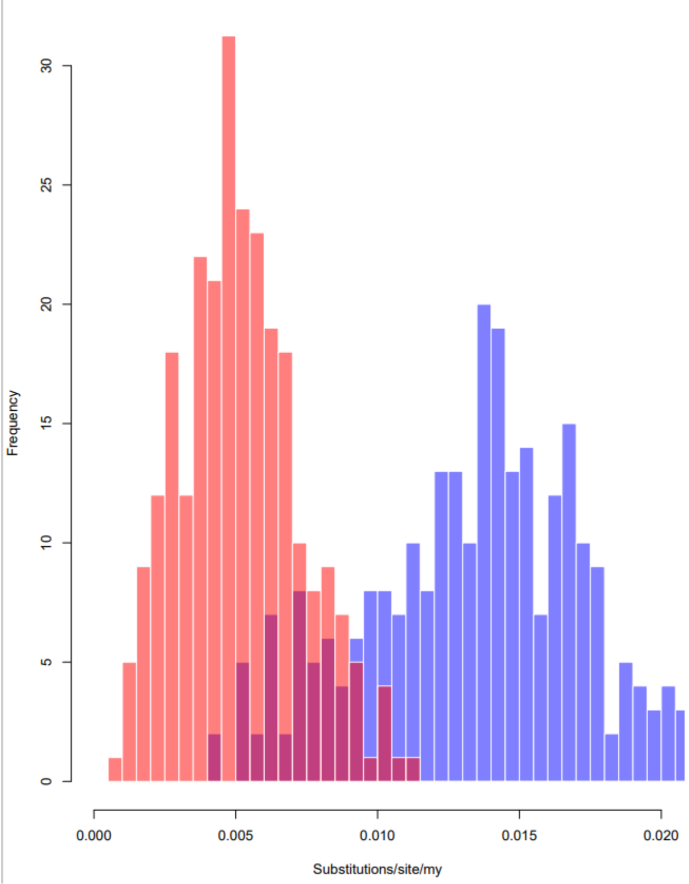

Figure 13. Histogram showing the difference in path branch length between the Malmeoideae (red) and the Annonoideae (blue). The dark red represents the overlap in path branch lengths between the Malmeoideae and the Annonoideae. The path branch lengths of the Annonoideae are larger than the path branch lengths of the Malmeoideae.

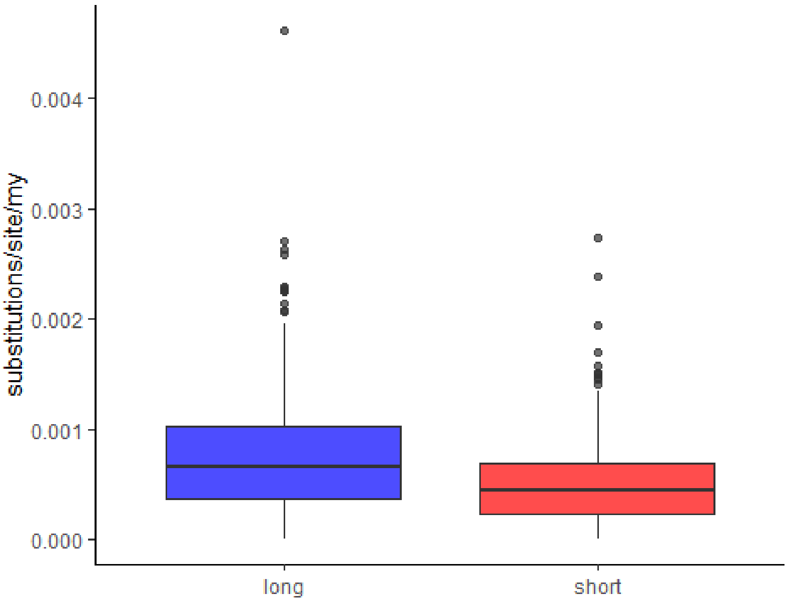

Figure 14. Boxplot showing difference in species branch length, the sum of the branch lengths from the crown node of the subfamily to each individual terminal. The path branch lengths of the Annonoideae are slightly larger than the path branch lengths of the Malmeoideae.

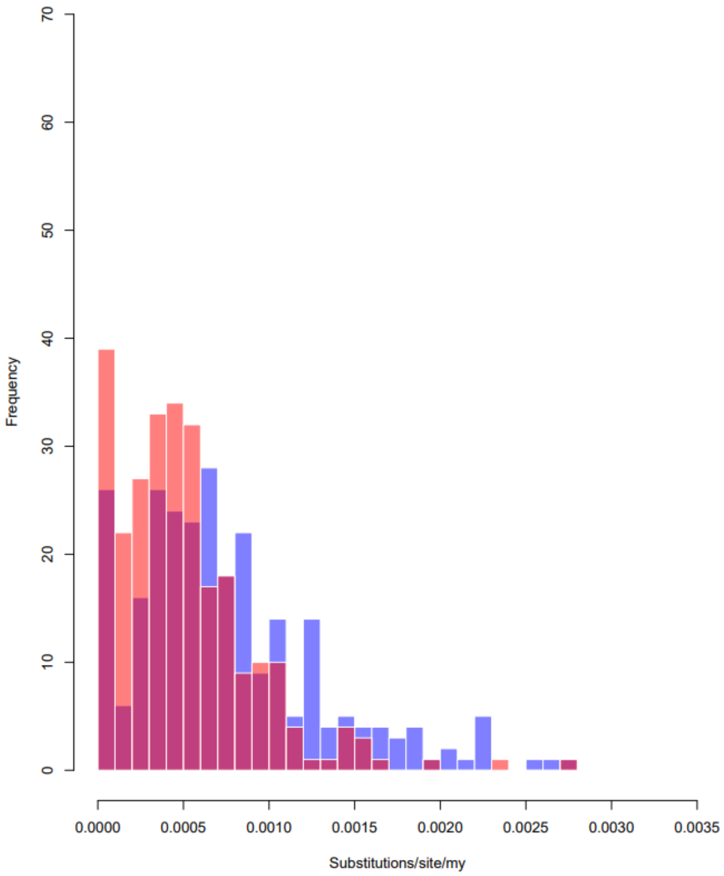

Figure 15. Histogram showing the difference in species branch length between the Malmeoideae (red) and the Annonoideae (blue). The dark red represents the overlap in path branch lengths between the Malmeoideae and the Annonoideae. The path branch lengths of the Annonoideae are slightly larger than the path branch lengths of the Malmeoideae.

Statistical analyses: Mortality rates

When analysing generation time, no significant differences (p-value > 0.05) were found between the Malmeoideae and Annonoideae. The mean generation time for the Annonoideae was 0.64 and for the Malmeoideae 0.76. To explain the variation we observe in the substitution rate, we analysed whether substitution rates are correlated with generation time. We found a significant (p-value < 0.05) negative correlation between the path branch length, a proxy for the substitution rate, and generation time (**Fig. 16**). Furthermore, we found a significant (p-value < 0.05) positive correlation between the path branch length and mortality rate (**Fig. 17**). This is as predicted as the generation time is equal to 1/mortality rate, thus inverting the negative correlation to a positive correlation between the two variables. For the species branch lengths similar results were found, demonstrating a significant (p-value < 0.05) negative correlation between the species branch length, a proxy for the substitution rate, and generation time and a significant (p-value < 0.05) positive correlation between the species branch length and mortality rate (**Figs. 18, 19**).

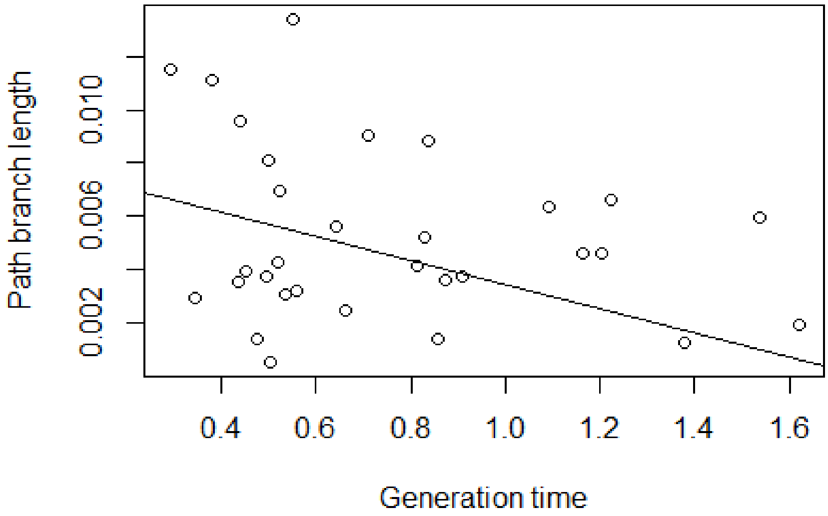

Figure 16. Results demonstrating a significant (p-value < 0.05) negative correlation between generation time and path branch length, a proxy for the substitution rate.

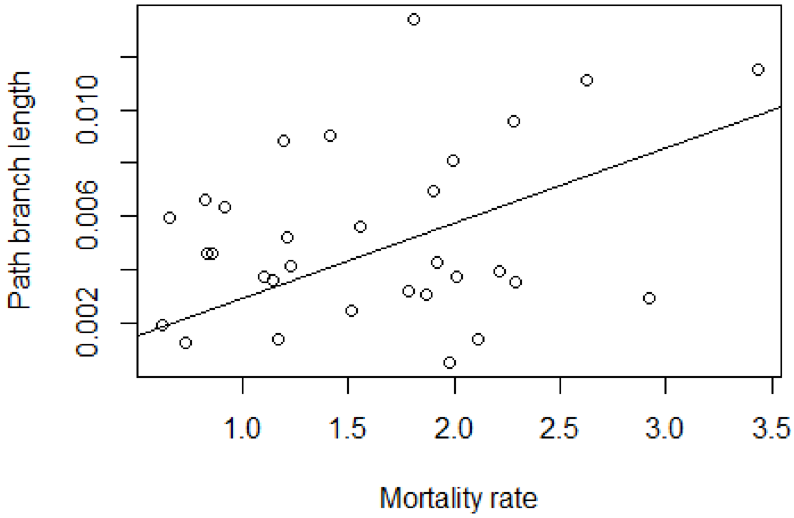

Figure 17. Results demonstrating a significant (p-value < 0.05) positive correlation between the mortality rate and path branch length, a proxy for the substitution rate.

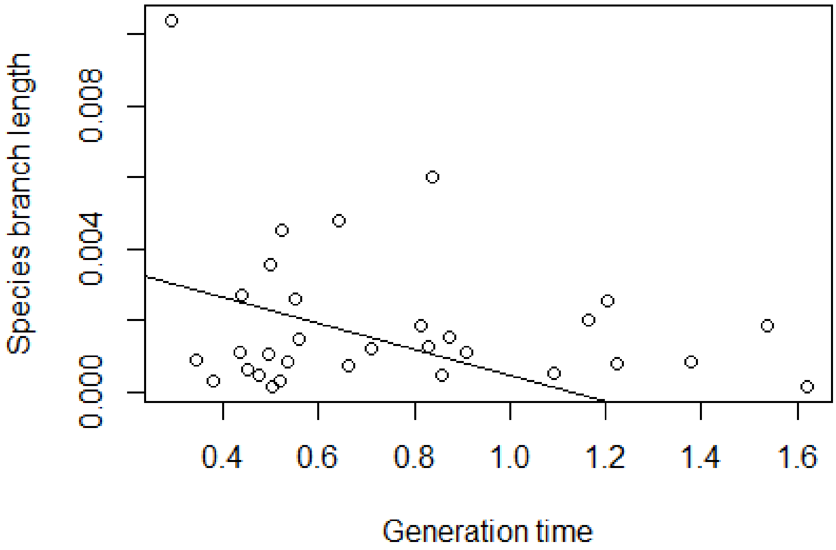

Figure 18. Results demonstrating a significant (p-value < 0.05) negative correlation between generation time and species branch length, a proxy for the substitution rate.

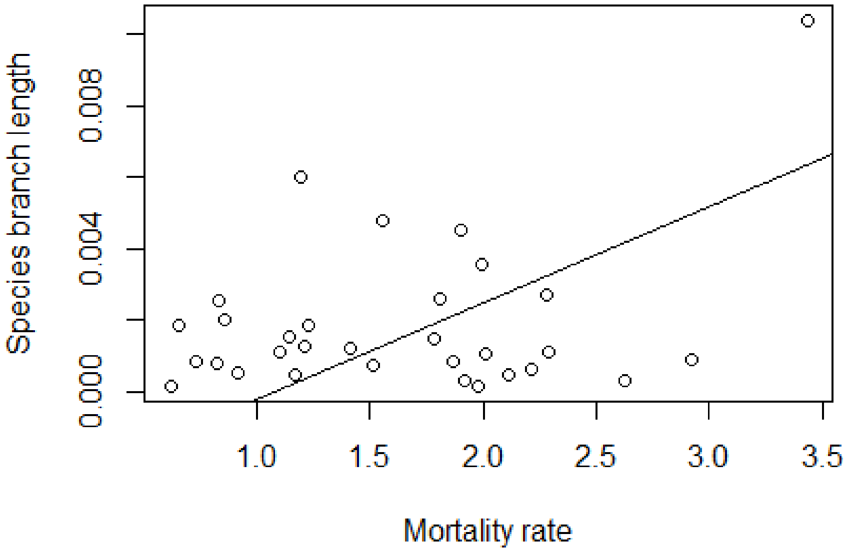

Figure 19. Results demonstrating a significant (p-value < 0.05) positive correlation between mortality rate and species branch length, a proxy for the substitution rate.

Discussion

This study clearly demonstrates that the path branch lengths and species branch lengths are significantly (p-value < 0.05) longer in the Annonoideae (the long branch clade) than in the Malmeoideae (the short branch clade) (**Figs. 12, 14**). Since we used the path branch length and species branch length as a proxy for the substitution rate, this study suggests faster or higher rates of substitution in the Annonoideae than in the Malmeoideae. Furthermore, contrary to our expectations, no significant differences (p-value > 0.05) were found in generation time between the Annonoideae and the Malmeoideae. Finally, we have found a significant (p-value < 0.05) negative correlation between generation time and substitution rate (**Figs. 16, 18**). Thus, first of all, our results confirm our hypotheses and the predictions, concerning the link between the branch length and substitution rate and substitution rate heterogeneity, made by different previous studies (Hoekstra et al., 2017; Kay and Hodges, 2006; Andreasen and Baldwin, 2001; Laroche and Bousquet, 1999; De La Torre et al., 2017). Second, our results confirm the generation time hypothesis (Li et al., 1987), which states that the shorter the generation time the more replication errors can be copied to the next generation and the higher the substitution rate.

According to earlier studies (Bromham et al., 1996; Bromham, 2011) these differences in rates of molecular evolution are driven by life-history traits such as body size, fecundity, generation time etc.. Several studies (Lanfear, 2013; Gaut et al., 1992; Smith and Donoghue, 2008; De La Torre et al., 2017) even suggested that this rate heterogeneity reflects differences in generation time between lineages. The generation time hypothesis states that a species with a shorter generation time accumulates more DNA replication errors because more germline-cell divisions occur per unit of time, thereby having higher mutation rates than a species with a longer generation time (Li et al., 1987). As mentioned before, plants lack a germline and their gametes are generated from the apical meristem only late in development. However, during periods of vegetative growth somatic mutations can occur, accumulate and be passed on to the next generation (Watson et al., 2016). Due to this, it was proposed (Lanfear et al., 2013) that differences in substitution rates can be accounted for by differences in the rate of mitotic cell divisions that occur before gametogenesis. Thus, according to this alternative generation time hypothesis, short-living plants, that flower early, have larger numbers of cell divisions, resulting in more opportunities to accumulate replication errors, and higher mutation rates. Since the generation time is defined by 1/mortality rate, the mortality rate was used as a proxy for the generation time in this study. We predicted that generation time is negatively correlated to the substitution rate, explaining why we observe higher rates of substitution in the Annonoideae compared to the Malmeoideae and our results confirmed those predictions.

As mentioned above, many life-history traits such as body size, metabolic rate, generation time etc. have been proposed as factors that drive the variation observed in the substitution rate (Bromham et al., 1996). It is very likely that a correlation between the substitution rate and a life-history trait could be influenced by other life-history traits or other factors. Therefore, it is important to assess whether the tested correlation is influenced by other factors or not. In previous studies (Lanfear et al., 2013; Bromham et al., 2015) it was suggested that taller plants have smaller substitution rates and these researchers also assessed whether the correlation of interest was influenced by other factors such as temperature, UV, latitude etc., as this is important for making correct conclusions. Environmental variables have been suggested as factors that could directly and indirectly drive rates of molecular evolution (Davies et al., 2004; Hawkins et al. 2003). An environmental factor that has an indirect effect is for example latitude (Brown, 2014). Latitude has an indirect effect on the substitution rate because as the latitude increases the temperature decreases which leads to less cell divisions per unit of time and lower substitution rates (De Frenne et al. 2013; Gillman et al. 2009; Gillman and Wright 2014). This effect is also observed for the altitude (Gillman et al., 2009). Further, UV has been proposed as a factor that directly affects the rate of substitution because of its mutagenic effect on the genome (Willis et al., 2009; Flenley, 2011). Since exposure towards UV increases with altitude we would expect higher substitution rates at higher altitudes, however several studies (Gillman et al., 2009; Wright et al., 2010) found lower substitution rates at higher altitudes. An explanation for the inverse correlation between UV and substitution rate could be that plants adjust their DNA repair mechanisms depending on the mutagen levels (Albarracín et al., 2012). Altogether, these studies demonstrate the importance of testing the effect of other variables on the correlation of interest. Since we used the molecular data from Genbank, we do not know anything about the location and environmental conditions where the species were sampled. Therefore, it is worth taking into account the possible effects of those environmental variables in future researches analysing the correlation between generation time and substitution rate.

No significant differences in generation time were found between the Annonoideae and Malmeoideae, which was in contrast to our expectations. Baker et al. (2017) indicated that there are differences in mortality rate between genera. However, this insignificance is probably due to the small dataset, which consisted of only 31 species of the Annonaceae, that was used to calculate the generation time. It is very likely that there would be significant differences in generation time between the two major clades if a larger dataset with more species would be used. Species sampling plays an important role in phylogenetic inference accuracy and consequently it can strongly affect the hypotheses that are being tested. The species names of all 819 species were critically controlled and if needed they were corrected according to KewScience (http://www.plantsoftheworldonline.org) and Annonbase (http://herbarium.botanik.univie.ac.at). However, it is important to keep in mind that some species could be incorrectly determined in the field and this could influence the phylogeny and analyses. Furthermore, the plant family Annonaceae consist of ca. 2500 species and this study used only 819 species. This could be another limitation influencing the results and conclusions made during this study.

Some studies (Jansen et al., 1991; Wallace and Jansen, 1990; Whittle and Johnston, 2003) contradict the generation time hypothesis because they did not find a correlation between generation time and substitution rate. However, it could be possible that some species belonging to the Malmeoideae (the short branch clade) coincidentally evolved faster than expected and vice versa for species of the Annonoideae (the long branch clade). Another explanation for the absence of a correlation between generation time and substitution rate is that the correlation of interest is confounded by other variables for example other life-history traits such as size, fecundity etc. or environmental factors as mentioned above. This thesis project underlines the need for more information and the understanding on the underlying drivers of variation in the rate of molecular evolution.

Conclusion

During this study we were able to demonstrate significant differences in path branch length and species branch length between the Annonoideae and the Malmeoideae, respectively known as the long branch clade and the short branch clade. Since the path branch length and the species branch length were used as proxies for the substitution rate, we could provide evidence and support for our first hypothesis which stated that significant differences in branch length reflect significant differences in substitution rate between the Annonoideae and the Malmeoideae. With these results we do not only support our hypothesis, but also the predictions made by previous studies (Richardson et al., 2004; Hoekstra et al., 2017). In contrast to our expectations, we found no significant differences in generation time between the Annonoideae and Malmeoideae, however this is probably due to the limited mortality rate dataset. Furthermore, this study found a significant negative correlation between generation time and substitution rate, providing evidence and support for the generation time hypothesis (Li et al., 1987). Our results suggest that the longer the generation time the less cell divisions occur per time unit, resulting in less opportunities to accumulate DNA replication errors and thus the lower the substitution rate or in other words the shorter the branch length. This is in line with our final hypothesis, which stated that there is a negative correlation between the generation time and the substitution rate, and many previous studies ((Smith and Donoghue, 2008; Laroch and Bousquet, 1999) that provide support for the generation time hypothesis.

Summary

For evolution to occur there must be genetic variation, but what drives genetic variation? The answer are mutations. During DNA replication mutations can occur, causing the formation of a new allele, which can be inherited by descendants. When this mutated allele becomes fixed, either by natural selection or chance, and is carried by all individuals of a population it is called a substitution. This evolutionary change of DNA sequences over time is known as molecular evolution. Early studies of molecular evolution suggested the molecular clock hypothesis, stating that the substation rate is constant. However, later, tremendous evidence against the molecular clock hypothesis arose, demonstrating variation in substitution rates at different levels. It has been proposed that this substitution rate heterogeneity is driven by several factors such as metabolic rate, environmental energy, generation time etc.. The generation time hypothesis states that a species with a shorter generation time copies its genome more often, resulting in more cell divisions per time unit, thereby having more opportunities to accumulate DNA replication errors compared to a species with a longer generation time. This study will explore the correlation between generation time and substitution rate among species of different clades of the flowering plant family Annonaceae and will test whether differences in generation time between species provide a good explanation for the substitution rate heterogeneity.

Molecular sequence data of 819 Annonaceae species were assembled using five different chloroplast markers: *matK*, *ndhF*, *psbA-trnH*, *rbcL* and *trnLF.* We aligned each used marker and concatenated the dataset into a single sequence per species. A Bayesian analysis was performed to obtain the phylogenetic tree. Each analysis ran for ca. 50 000 000 generations with two parallel runs, each with four independent Metropolis-Coupled Markov Chain Monte Carlo chains. Two fossils, *Endressinia brasiliana* and *Futabanthus asamigawaensis,* were used to calibrate the inferred phylogenetic tree. To test the correlation between generation time and substitution rate, two proxies were used respectively: mortality rate and branch length. We divided the branch lengths of the phylogram (measured in substitutions/site) by the branch lengths of the chronogram (measured in million years) to obtain branch lengths measured in substitutions per site per million years. First, we tested whether the branch lengths of the subfamily Annonoideae were significantly longer than the branch lengths of the subfamily Malmeoideae by performing a Wilcoxon rank sum test in RStudio. We used two different measures for the branch length: the path branch length, which is the sum of the branch lengths from the crown node of the subfamily to each terminal, and the species branch length, which is the terminal branch length for each species. Second, we tested whether there were significant differences in generation time, for which mortality rate was used as proxy, between the Annonoideae and the Malmeoideae by using a Wilxocon rank sum test in RStudio. At last, we evaluated the correlation between generation time and substitution rate by using (Phylogenetic) Generalized Least Squares (pgls and gls) in RStudio.

Our results demonstrated that the path branch lengths and species branch lengths are significantly (p-values < 0.05) longer in the Annonoideae compared to the path branch lengths and the species branch lengths of the Malmeoideae. Since branch length is used as a proxy for the substitution rate, our results suggest significantly higher substitution rates in the Annonoideae. No significant differences (p-value > 0.05) in generation time were found between the Annonoideae and the Malmeoideae. Finally, a significant (p-value < 0.05) negative correlation between generation time and substitution rate was found. This study has demonstrated for the first time that generation time is a factor driving the

variation in substation rates in species of the pantropical plant family Annonaceae. However, other factors such as body size, fecundity, environmental factors etc. have also been proposed as drives of the substitution rate heterogeneity. Therefore, it is important for future researches to take these factors into account and test whether there are factors that influence the correlation and hypotheses that are being tested. No significant differences in generation time were found, which is probably due to the limited dataset that included only 31 species. Furthermore, it is important to keep in mind that errors made concerning the species sampling could affect the results and conclusions. We can conclude that the branch lengths or substitution rates are higher in the Annonoideae compared to the Malmeoideae as was predicted by previous studies. Second, we could not find significant differences in generation time between the two major subfamilies. At last, this study demonstrated a significant negative correlation between generation time and substitution rates among species of the Annonaceae, thereby supporting the generation time hypothesis.

Samenvatting

Opdat evolutie kan plaatsvinden, moet er genetische variatie zijn, maar wat drijft genetische variatie? Het antwoord hierop zijn mutaties. Tijdens DNA replicatie kunnen mutaties plaatsvinden en dit kan leiden tot de vorming van een nieuw allel dat op zijn beurt kan overgeërfd worden door de nakomelingen. Indien het gemuteerde allel, door ofwel natuurlijke selectie of toeval, na verloop van tijd gefixeerd wordt en gedragen wordt door elk individu binnen de populatie, dan wordt dit een substitutie genoemd. Het proces waarbij DNA sequenties veranderen na verloop van tijd wordt moleculaire evolutie genoemd. De eerste studies binnen het domein van de moleculaire evolutie stelden de moleculaire klok hypothese voor. Volgens deze hypothese is de snelheid waaraan substituties plaatsvinden constant. Echter, door de jaren heen, verzamelde men bewijs tegen de moleculaire klok hypothese waarbij men aantoonde dat er wel degelijk variatie is in de substitutiesnelheid. Men veronderstelt dat deze variatie in substitutiesnelheid beïnvloed wordt door verschillende factoren zoals het metabolisme, de omgeving, generatietijd etc.. De generatietijd hypothese verklaart dat een soort met een korte generatietijd vaker zijn genoom kopieert waardoor veel meer celdelingen plaatsvinden en dit vergroot de kans op het verzamelen van mutaties of fouten tijdens de DNA replicatie in vergelijking met een soort dat een lange generatietijd heeft. Deze studie zal de correlatie tussen de generatietijd en de substitutiesnelheid onderzoeken voor verschillende soorten van de plantenfamilie Annonaceae en zal bepalen of het verschil in generatietijd tussen soorten de variatie in substitutiesnelheid kan verklaren.

De moleculaire DNA sequentie van 819 soorten van de plantenfamilie Annonaceae werd verzameld door middel van vijf verschillende chloroplast markers: matK, ndhF, psbA-trnH, rbcL and trnLF. Elke marker werd uitgelijnd en de dataset werd samengevoegd. Een Bayesiaanse analyse werd uitgevoerd om de fylogenetische boom te bekomen. Elke analyse duurde ca. 50 000 000 generaties en steeds twee analyses verliepen simultaan met telkens elk 4 onafhankelijke Metropolis-Coupled Markov Chain Monte Carlo chains. Twee fossielen, Endressinia brasiliana and Futabanthus asamigawaensis, werden gebruikt voor het kalibreren van de fylogenetische boom. Om de correlatie tussen de generatietijd en de substitutiesnelheid te testen werden respectievelijk 2 proxy's gebruikt: de mortaliteit en de tak lengte. We hebben de tak lengte van de fylogenetische boom (uitgedrukt in substitutiesnelheid/site) gedeeld door de tak lengte van de gekalibreerde fylogenetische boom (uitgedrukt in miljoen jaar) zodat we tak lengtes verkregen die worden uitgedrukt in substitutiesnelheid per site per miljoen jaar. Eerst hebben we onderzocht of de tak lengtes van de subfamilie Annonoideae significant langer zijn dan de tak lengtes van de subfamilie Malmeoideae door middel van een Wilcoxon rank sum test in RStudio. We hebben twee verschillende proxy's gebruikt voor de tak lengte: de pad tak lengte, dit is de som van de tak lengtes gaande van de oorsprong van de subfamilie tot elke soort, en de soort tak lengte, de effectieve tak lengte van elke soort. Vervolgens hebben we getest of de generatietijd, waarvoor we als proxy de mortaliteit gebruiken, significant verschilt tussen de Annonoideae en de Malmeoideae door middel van een Wilcoxon rank sum test in RStudio. Tot slot hebben we de correlatie tussen de generatietijd en substitutiesnelheid onderzocht door middel van statistische functies in RStudio.

Onze resultaten tonen aan dat de pad tak lengtes en de soort tak lengtes significant langer zijn in de Annonoideae in vergelijking met deze van de Malmeoideae. Aangezien we de tak lengte gebruiken als proxy voor de substitutiesnelheid kunnen we concluderen dat de substitutiesnelheid significant hoger is in de Annonoideae. Geen significante verschillen in generatietijd werden vastgesteld tussen de Annonoideae en de Malmeoideae. Tot slot werd een significant negatieve correlatie tussen de

generatietijd en de substitutiesnelheid vastgesteld tijdens deze studie. Dit onderzoek toont voor de eerste keer aan dat de generatietijd een belangrijke factor is die de variatie in substitutiesnelheid binnen de plantenfamilie Annonaceae beïnvloedt. Naast de generatietijd zijn ook andere factoren zoals de vruchtbaarheid, lichaamsgrootte, latitude, UV etc. voorgesteld als factoren die de variatie in substitutiesnelheid verklaren. Daarom is het zeer belangrijk dat toekomstige studies die de correlatie tussen generatietijd en substitutiesnelheid willen onderzoeken, rekening houden met dergelijke factoren aangezien deze een invloed kunnen hebben op de correlatie. Bij het analyseren van de generatietijd werden geen significante verschillen gevonden. Dit is hoogstwaarschijnlijk te wijten aan de beperkte gegevens aangezien de mortaliteit voor slechts 31 soorten werd verzameld. Daarnaast is het belangrijk om rekening te houden met het feit dat fouten, die worden gemaakt met betrekking tot de soortenbemonstering, de resultaten en conclusies kunnen beïnvloeden. Algemeen kunnen we uit deze studie concluderen dat de tak lengtes/substitutiesnelheden hoger zijn in de Annonoideae in vergelijking met de Malmeoideae en dit is in overeenstemming met wat was voorspeld door voorgaande studies. Vervolgens kon deze studie geen significante verschillen in generatietijd vaststellen. Tot slot heeft deze studie een significant negatieve correlatie tussen de generatietijd en substitutiesnelheid kunnen vaststellen en hierdoor bevestigt deze studie de generatietijd hypothese.

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Appendix

A. Table of species and accession numbers

Species name	Accession number	MATK	NDHF	PSBA_TRNH	RBCL	TRNLF
Alphonsea boniana	JB57	/	/	/	JB57	JB57
Alphonsea elliptica	JB34	JB34	JB34	JB34	JB34	JB34
Alphonsea kinabaluensis	JB85	/	/	/	JB85	JB85
Alphonsea sp	PK3186JB	PK3186JB	PK3186JB	PK3186JB	/	PK3186JB
Ambavia gerrardii	PB	/	/	/	PB	PB
Anaxagorea javanica	JB	/	/	/	JB	JB
Anaxagorea luzonensis	JB	JB	JB	JB	JB	JB
Anaxagorea phaeocarpa	498	498	498	498	498	498
Anaxagorea silvatica	113	113	113	113	113	113
Annickia affinis	976	976	976	976	976	976
Annickia_ambigua	?	?	?	?	?	?
Annickia chlorantha	976	976	976	976	976	976
Annickia kummeriae	MWC7004	/	/	/	MWC7004	/
Annickia_letestui	?	?	?	?	?	?
Annickia pilosa	977	977	977	977	977	977
Annickia_polycarpa	?	?	?	?	?	?
Annona amazonica	15	/	/	/	15	15
Annona bahiensis	1085	/	/	/	1085	1085
Annona bicolor	499	/	/	/	499	499
Annona cordifolia	324	/	/	/	324	324
Annona cornifolia	135	/	/	/	135	/
Annona cuspidata	185	/	/	/	185	185
Annona deceptrix	480	/	/	/	480	480
Annona dioica	728	/	/	/	728	728
Annona dumetorum	504	/	/	/	504	504
Annona edulis	297	/	/	/	297	297
Annona flava	137	/	/	/	137	137
Annona foetida	133	/	/	/	133	133
Annona glabra	522	522	522	522	522	522
Annona herzogii	1051	1051	1051	1051	1051	1051
Annona holosericea	500	/	/	/	500	500
Annona hypoglauca	140	/	/	/	140	140
Annona macroprophyllata	523	/	/	/	523	523
Annona montana	524	/	/	/	524	524
Annona mucosa	325	/	/	/	325	325
Annona muricata	525TC	525TC	525TC	525TC	525TC	525TC
Annona neochrysocarpa	1120	/	/	/	1120	1120
Annona oligocarpa	1084	/	/	/	1084	1084
Annona pruinosa	229	/	/	/	229	229
Annona reticulata	118	/	/	/	118	118
Annona scandens	141	/	/	/	141	141
Annona senegalensis	527	/	/	/	527	527

Annona sericea	117	/	/	/	117	/
Annona squamosa	506	/	/	/	506	506
Annona symphyocarpa	48	/	/	/	48	48
Annona urbaniana	488	/	/	/	488	488
Anonidium sp	38	38	38	38	38	38
Artabotrys_aurantiacus	?	KM924834	KM924864	KM924965	KM924904	KM924932
Artabotrys_carnosipetalus	?	KM924835	KM924865	KM924966	KM924905	KM924933
Artabotrys_crassifolius	?	KM924836	KM924866	KM924967	KM924906	KM924934
Artabotrys_gracilis	?	KM924837	KM924867	KM924968	KM924907	KM924935
Artabotrys_harmandii	?	KM924838	KM924868	KM924969	KM924908	KM924936
Artabotrys hexapetalus	0529	AY238962	EF179284	AY841429	AY238953	AY231286
Artabotrys_hongkongensis	?	KM924839	KM924869	KM924970	KM924909	KM924937
Artabotrys_longistigmatus	?	KM924840	KM924870	KM924971	KM924910	KM924938
Artabotrys_madagascariensis	?	/	KM924871	KM924972	/	KM924939
Artabotrys_modestus	?	KM924841	KM924872	KM924973	KM924911	KM924940
Artabotrys_monteiroae	?	KM924842	KM924873	KM924974	KM924912	KM924941
Artabotrys_pierreanus	?	KM924843	KM924874	KM924975	KM924913	KM924942
Artabotrys sp	0121	0121	0121	0121	0121	121
Artabotrys spec Gabon	GB	DQ125052	EF179285	DQ125118	AY841599	AY841676
Artabotrys_sp_PNG	?	KM924844	KM924875	KM924976	KM924914	KM924943
Artabotrys_sp_Sumatra	?	KM924845	KM924876	KM924977	KM924915	KM924944
Artabotrys_stolzii	?	KM924846	KM924877	KM924978	KM924916	KM924945
Artabotrys_thomsonii	?	KM924847	KM924878	KM924979	KM924917	KM924946
Artabotrys_velutinus	?	KM924848	KM924879	KM924980	KM924918	KM924947
Asimina angustifolia	1076	1076	1076	1076	1076	1076
Asimina_rugelii_KN	?	?	?	?	?	?
Asimina triloba	0517	0517	0517	0517	0517	0517
Asteranthe asterias	TC35	TC35	TC35	TC35	TC35	TC35
Bocageopsis canescens	TNW106	TNW106	TNW106	TNW106	TNW106	TNW106
Bocageopsis mattogrossensis	1318	1318	/	1318	1318	1318

Bocageopsis multiflora	0965	0965	0965	0965	0965	0965
Bocageopsis pleiosperma	0332	0332	/	0332	PB	PB
Cananga odorata	0239	0239	0239	0239	0239	0239
Cleistopholis glauca	0198	0198	0198	0198	0198	0198
Cremastosperma brevipes	0601	0601	0601	0601	0601	0601
Cremastosperma megalophyllum	0493	/	/	/	0493	0493
Crematosperma brevipes	0601	0601	0601	0601	0601	0601
Crematosperma bullatum	1100	1100	1100	1100	1100	1100
Crematosperma cauliflorum	0312	0312	0312	0312	0312	0312
Crematosperma confusum	0756	0756	0756	0756	0756	0756
Crematosperma gracilipes	0491	0491	0491	0491	0491	0491
Crematosperma leiophyllum	0594	0594	0594	0594	0594	0594
Crematosperma longicuspe	0745	0745	0745	0745	0745	0753
Crematosperma macrocarpum	0741	0741	0741	0741	0741	0741
Crematosperma magdalenae	1270	1270	/	1270	1270	1270
Crematosperma megalophyllum	0490	0490	0490	0490	0490	0490
Crematosperma microcarpum	0303	0303	0303	0303	0303	0303
Crematosperma monospermum	0596	0596	0596	0596	0596	0596
Crematosperma napoense	0737	0737	0737	0737	0737	0737
Crematosperma novogranatense	0744	0744	/	0744	0744	0744
Crematosperma oblongum	0739	0739	0739	0739	0739	0739
Crematosperma osicola	0245	0245	0245	0245	0245	0245
Crematosperma panamense	0747	0747	0747	0747	0747	0747
Crematosperma pedunculatum	0760	0760	0760	0760	0760	0760
Crematosperma pendulum	0751	0751	0751	0751	0751	0751
Crematosperma peruvianum	0755	0755	0755	0755	0755	0755
Crematosperma sp	0765	0765	/	0765	0765	0765
Crematosperma stenophyllum	0767	0767	0767	0767	0767	0767

Crematosperma venezuelanum	0757	0757	0757	0757	0757	0757
Crematosperma westrae	0761	0761	0761	0761	0761	0761
Crematosperma yamayacatense	1099	1099	1099	1099	1099	1099
Cyathocalyx biovulatus	MWC7015	/	/	/	MWC7015	MWC7015
Cyathocalyx martabanicus	1002	1002	1002	1002	1002	1002
Cymbopetalum brasiliense	0530	0530	0530	0530	0530	0530
Cymbopetalum sp	0098	/	/	/	0098	0098
Cymbopetalum torulosum	0210	/	/	/	0210	0210
Dasymaschalon acuminatum	?	?	?	/	?	/
Dasymaschalon borneense	?	?	/	/	?	/
Dasymaschalon clusiflorum	?	?	/	/	?	/
Dasymaschalon dasymaschalum	?	?	?	?	?	?
Dasymaschalon_dasymaschalus	?	?	?	?	?	?
Dasymaschalon ellipticum	?	?	?	/	?	/
Dasymaschalon evrardii	?	?	/	/	?	/
Dasymaschalon filipes	?	?	/	/	?	/
Dasymaschalon glaucum	?	?	?	/	?	/
Dasymaschalon lomentaceum	?	?	?	?	?	?
Dasymaschalon longiflorum	?	?	?	/	?	?
Dasymaschalon longiusculum	?	?	/	/	?	/
Dasymaschalon macrocalyx	0997	0997	0997	0997	0997	0997
Dasymaschalon megalanthum	?	?	?	/	?	/
Dasymaschalon oblongatum	?	?	?	/	?	/
Dasymaschalon obtusipetalum	?	?	?	/	?	/
Dasymaschalon robinsonii	?	?	/	/	?	/
Dasymaschalon rostratum	?	?	?	/	?	/
Dasymaschalon sootepense	998	?	?	/	0998	0998
Dasymaschalon tibetense	?	?	?	/	?	/

Dasymaschalon trichophorum	?	?	?	/	?	/
Dasymaschalon wallichii	?	?	/	/	?	/
Dendrokingstonia gardneri	TNW3	TNW3	TNW3	TNW3	TNW3	TNW3
Dendrokingstonia nervosa	TNWH15	TNWH15	TNWH15	TNWH15	/	TNWH15
Desmopsis microcarpa	0234	0234	0234	0234	0234	0234
Desmopsis schippii	0240	/	/	/	0240	0240
Desmos chinensis	?	?	?	?	?	?
Desmos cochinchinensis	?	?	?	?	?	?
Desmos dinhensis	?	?	?	?	/	?
Desmos dumosus	?	?	?	/	?	?
Desmos elegans	Saunders	Saunders	/	/	Saunders	Saunders
Desmos goezeamos	?	?	?	/	?	/
Desmos wardianus	?	?	?	/	?	/
Diclinanona calycina	?	?	/	?	?	?
Diclinanona tessmannii	0076	?	0076	0076	0076	0076
Dielsiothamnus divaricatus	TC129	TC129	?	TC129	TC129	TC129
Disepalum pulchrum	KN	/	/	/	KN	KN
Disepalum sp	1077	1077	1077	1077	1077	1077
Duckeanthus grandiflorus	878	878	/	/	878	878
Duguetia bahiensis	0475	0475	/	0475	0475	0475
Duguetia cadaverica	0339	0339	/	0339	0339	0339
Duguetia calycina	0348	0348	/	0348	0348	0348
Duguetia cauliflora	0357	0357	/	0357	0357	0357
Duguetia chrysea	0362	0362	/	0362	0362	0362
Duguetia confinis	0365	0365	/	0365	0365	0365
Duguetia confusa	0201	0201	/	0201	0201	0201
Duguetia echinophora	0372	0372	/	0372	0372	0372
Duguetia flagellaris	0378	0378	/	0378	0378	0378
Duguetia furfuracea	0846	0846	/	0846	0846	0846
Duguetia guianensis	0153	0153	/	0153	0153	0153
Duguetia hadrantha	0288	0288	0288	0288	0288	0288
Duguetia inconspicua	0390	0390	/	0390	0390	0390
Duguetia lepidota	0397	/	/	0397	/	0397
Duguetia lucida	0732	0732	/	0732	0732	0732
Duguetia macrocalyx	0401	0401	/	0401	0401	0401
Duguetia macrophylla	0403	0403	/	0403	0403	0403
Duguetia marcgraviana	0729	0729	/	0729	0729	0729
Duguetia megalocarpa	0406	0406	/	0406	0406	0406
Duguetia moricandiana	0408	0408	/	0408	0408	0408
Duguetia neglecta	0410	0410	/	0410	0410	0410
Duguetia odorata	0302	0302	/	0302	0302	0302
Duguetia panamensis	0242	0242	/	0242	0242	0242
Duguetia peruviana	0476	0476	/	0476	0476	0476
Duguetia pycnastera	0416	0416	/	0416	0416	0416

Duguetia quitarensis	0261	0261	/	0261	0261	0261
Duguetia riedeliana	0424	0424	/	0424	0424	0424
Duguetia riparia	0434	0434	/	0434	0434	0434
Duguetia rotundifolia	1415	1415	/	1415	1415	1415
Duguetia salicifolia	0426	0426	/	0426	0426	0426
Duguetia sessilis	0427	0427	/	0427	0427	0427
Duguetia sooretamae	0428	0428	/	0428	0428	0428
Duguetia spixiana	281734	281734	/	281734	281734	281734
Duguetia staGB	?	?	/	?	/	?
Duguetia staudtii	0854	0854	0854	0854	0854	0854
Duguetia stelechantha	0451	0451	/	0451	0451	0451
Duguetia surinamensis	0455	0455	/	0455	0455	0455
Duguetia ulei	0335	0335	/	0335	0335	0335
Duguetia uniflora	0457	0457	/	0457	0457	0457
Duguetia yeshidan	0461	0461	/	0461	0461	0461
Ephedranthus amazonicus	0284	?	?	?	0284	0284
Ephedranthus boliviensis	1036	?	?	?	1036	1036
Ephedranthus dimerus	0105	0105	0105	0105	0105	0105
Ephedranthus parviflorus	0031	0031	/	0031	0031	0031
Fenerivia chapelieri	TNW36	TNW36	TNW36	TNW36	TNW36	TNW36
Fenerivia_angustielliptica	?	AY841396	/	/	AY841616	AY841694
Fenerivia_capuronii	?	JF810373	/	/	JF810385	JF810397
Fenerivia_emarginata	?	JF810376	/	/	JF810388	JF810400
Fenerivia ghesquiereana	TNW37	TNW37	TNW37	TNW37	TNW37	TNW37
Fenerivia_heteropetala	?	JF810389	/	/	JF810377	JF810401
Fenerivia_humbertii	?	JF810378	/	/	JF810390	JF810402
Fenerivia_madagascariensis	?	JF810379	/	/	JF810391	JF810403
Fenerivia_oligosperma	?	JF810380	/	/	JF810392	JF810404
Fenerivia_richardiana	?	JF810381	/	/	JF810393	JF810405
Fissistigma bicolor	?	/	?	?	/	?
Fissistigma glaucescens	?	?	?	?	?	?
Fissistigma kingii	?	/	/	/	/	?
Fissistigma polyanthoides	?	?	/	/	/	?
Fissistigma uonicum	1021	/	/	/	1021	1021
Fitzalania bidwillii	TNWH55	TNWH55	TNWH55	TNWH55	/	TNWH55
Fitzalania heteropetala	MWC7030	TNW_H54	TNW_H54	TNW_H54	MWC7030	TNW_H54
Friesodielsia desmoides	?	?	?	?	?	?
Fusaea longifolia	0634	/	/	/	0634	0634

Fusaea peruviana	0287	0287	0287	0287	0287	0287
Goniothalamus_amuyon	?	KM818567	KM818648	KM818728	KM818839	KM818898
Goniothalamus_andersonii	?	KM818568	/	KM818711	KM818789	KM818867
Goniothalamus_angustifolius	?	KM818569	KM818632	KM818732	KM818797	KM818878
Goniothalamus_aruensis	?	KM818570	KM818640	KM818706	KM818791	KM818868
Goniothalamus_australis	?	KM818571	KM818638	KM818709	KM818836	KM818887
Goniothalamus_borneensis	?	KM818572	KM818673	/	KM818826	KM818893
Goniothalamus_bracteosus	?	KM818573	/	KM818730	KM818796	KM818906
Goniothalamus_calcareus	?	/	/	KM818717	KM818810	/
Goniothalamus_calvicarpus	?	KM818574	KM818647	KM818672	KM818809	KM818874
Goniothalamus_cardiopetalus	?	KM818575	KM818654	KM818692	KM818799	KM818879
Goniothalamus_cauliflorus	?	KM818576	KM818663	KM818696	KM818807	KM818869
Goniothalamus_cheliensis	?	KM818577	KM818661	KM818678	KM818831	KM818901
Goniothalamus_clemensii	?	KM818578	/	KM818736	KM818780	KM818844
Goniothalamus_costulatus	?	KM818579	/	KM818737	KM818805	KM818865
Goniothalamus_dumontetii	?	KM818580	/	KM818729	KM818840	KM818861
Goniothalamus_elegans	?	KM818581	KM818676	KM818707	KM818817	KM818850
Goniothalamus_elmeri	?	KM818582	KM818639	KM818677	KM818811	KM818882
Goniothalamus_expansus	?	KM818583	KM818634	KM818714	KM818829	KM818853
Goniothalamus_fasciculatus	?	KM818584	KM818636	/	/	KM818890
Goniothalamus_gardneri	?	KM818585	KM818656	KM818704	KM818784	KM818871
Goniothalamus_giganteus	?	KM818586	KM818655	KM818698	KM818837	KM818892
Goniothalamus_grandiflorus	?	KM818587	KM818637	KM818691	KM818802	KM818851
Goniothalamus griffithii	1019	KM818588	KM818651	KM818701	KM818798	KM818894
Goniothalamus_hookeri	?	KM818589	KM818657	KM818734	KM818814	KM818872
Goniothalamus_howii	?	KM818590	/	KM818689	KM818833	KM818886

Goniothalamus_imbricatus	?	KM818591	/	KM818722	KM818806	KM818847
Goniothalamus_kinabaluensis	?	KM818592	KM818672	KM818684	KM818787	KM818876
Goniothalamus laoticus	1018	KM818593	KM818666	KM818699	KM818808	KM818881
Goniothalamus_loerzingii	?	KM818594	/	KM818724	KM818782	KM818902
Goniothalamus_macranthus	?	KM818595	KM818643	KM818695	KM818792	KM818873
Goniothalamus_macrophyllus	?	KM818596	KM818665	KM818688	KM818843	KM818897
Goniothalamus_maewongensis	?	KM818597	KM818659	KM818725	KM818838	KM818888
Goniothalamus_majestatis	?	KM818598	/	KM818713	KM818788	KM818903
Goniothalamus_malayanus	?	KM818599	KM818650	KM818718	KM818835	KM818891
Goniothalamus_megalocalyx	?	KM818600	KM818645	KM818726	KM818822	KM818885
Goniothalamus_monospermus	?	KM818600	/	KM818735	KM818790	/
Goniothalamus_montanus	?	KM818602	KM818674	KM818710	KM818813	KM818856
Goniothalamus_obtusatus	?	KM818603	KM818660	KM818687	KM818815	KM818883
Goniothalamus_palawanensis	?	KM818604	/	KM818716	KM818793	KM818855
Goniothalamus_parallelovenius	?	KM818605	KM818635	KM818683	KM818801	KM818880
Goniothalamus_repevensis	?	KM818606	KM818664	KM818723	KM818795	KM818877
Goniothalamus_reticulatus	?	KM818607	/	/	KM818786	/
Goniothalamus_ridleyi	?	KM818608	/	KM818739	KM818830	KM818860
Goniothalamus_rotundisepalus	?	KM818609	KM818649	KM818693	KM818794	KM818857
Goniothalamus_rufus	?	KM818610	/	KM818727	KM818819	KM818848
Goniothalamus_sawtehii	?	KM818611	KM818646	KM818680	KM818785	KM818895
Goniothalamus_scortechinii	?	KM818612	KM818670	KM818712	KM818781	KM818845
Goniothalamus_sesquipedalis	?	KM818613	KM818667	KM818719	KM818825	KM818904
Goniothalamus_sp_tcc10	?	KM818614	KM818675	KM818715	KM818821	KM818864
Goniothalamus_suaveolens	?	KM818616	/	KM818682	KM818800	KM81885

Goniothalamus_tamirensis	?	KM818617	KM818662	KM818700	KM818832	KM818866
Goniothalamus tapis	1017	1017	1017	1017	1017	1017
Goniothalamus_tapisoides	?	KM818618	KM818641	KM818686	KM818823	KM818899
Goniothalamus_tavoyensis	?	KM818619	KM818633	KM818690	KM818841	KM818854
Goniothalamus_tenuifolius	?	KM818620	KM818669	KM818694	KM818842	KM818889
Goniothalamus_thomsonii	?	KM818621	/	KM818733	KM818834	KM818875
Goniothalamus_thwaitesii	?	KM818622	KM818653	KM818703	/	KM818849
Goniothalamus_tomentosus	?	KM818623	/	KM818738	KM818783	KM818846
Goniothalamus_tortilipetalus	?	KM818624	KM818642	KM818708	KM818828	KM818905
Goniothalamus_touranensis	?	KM818625	/	KM818731	KM818804	KM818870
Goniothalamus_undulatus	?	KM818626	KM818652	KM818679	KM818820	KM818896
Goniothalamus_uvarioides	?	KM818627	KM818658	KM818685	KM818827	KM818852
Goniothalamus_velutinus	?	KM818628	KM818644	KM818705	KM818812	KM818900
Goniothalamus_woodii	?	KM818629	KM818668	KM818720	KM818824	KM818862
Goniothalamus_wrayi	?	KM818630	KM818671	KM818721	KM818803	KM818859
Goniothalamus_wynaadensis	?	KM818631	/	KM818697	KM818816	KM818863
Greenwayodendron oliveri	0958	0958	0958	0958	0958	0958
Greenwayodendron suaveolens	MWC7037	/	/	/	MWC7037	MWC7037
Guamia mariannae	TNW92	TNW92	TNW92	TNW92	TNW92	TNW92
Guatteria aberrans	1321	1321	/	1321	1321	1321
Guatteria aeruginosa	0221	0221	0221	0221	0221	0221
Guatteria aff trichoclonia	AL731	AL_731	PF2591	/	AL_731	AL_731
Guatteria alata	776	776	/	776	776	776
Guatteria allenii	777	777	/	777	777	777
Guatteria alta	841	841	/	841	841	841
Guatteria alutacea	578	578	/	578	578	578
Guatteria amplifolia	220	220	/	220	220	220
Guatteria anomala	0150	0150	0150	0150	0150	0150
Guatteria argentea	829	829	/	829	829	829
Guatteria asterantha	AL	/	AL	/	AL	AL
Guatteria atra	715	715	/	715	715	715
Guatteria australis	0512	0512	AL646	0099	0512	0512
Guatteria blainii	785	785	/	785	785	785

Guatteria blepharophylla	323	323	/	323	323	323
Guatteria boliviana	838	838	/	838	838	838
Guatteria brevicuspis	801	801	/	801	801	801
Guatteria brevipedicellata	925type	925type	/	925type	925type	925type
Guatteria burchellii	AL1356	AL1356	AL1356	AL1356	AL1356	/
Guatteria candolleana	798	798	/	798	798	798
Guatteria caribaea	786	786	/	786	786	786
Guatteria chiriquiensis	202	202	/	202	202	202
Guatteria citriodora	696	696	/	696	696	696
Guatteria decurrens	0555	0555	/	0295	0555	0555
Guatteria diospyroides	0233	0233	/	0233	0233	0233
Guatteria discolor	552	552	/	552	552	552
Guatteria dolichopoda	1346	1346	/	1346	1346	1346
Guatteria dumetorum	779	779	/	779	779	779
Guatteria elata	328	328	/	328	328	328
Guatteria elegantissima	833	833	/	833	833	833
Guatteria ferruginea	565	565	/	565	565	565
Guatteria foliosa	0575	0575	/	0575	0575	0575
Guatteria galleotiana	1404	1404	/	1404	1404	1404
Guatteria guianensis	560	560	/	560	560	560
Guatteria hirsuta	1199	1199	/	0827	1199	1199
Guatteria inundata	291	291	/	291	291	291
Guatteria jefensis	781	781	/	781	781	781
Guatteria latifolia	510	510	/	510	510	510
Guatteria liesneri	792	792	/	792	792	792
Guatteria lucens	782	782	/	782	782	782
Guatteria macropus	790	790	/	790	790	790
Guatteria maypurensis	182	182	/	182	182	182
Guatteria megalophylla	309	309	/	309	309	309
Guatteria modesta	823	823	/	823	823	823
Guatteria notabilis	567	567	/	567	567	567
Guatteria oligocarpa	811	811	/	811	811	811
Guatteria ouregou	679	679	/	679	679	679
Guatteria pacifica	887type	887type	/	887type	887type	887type
Guatteria paraensis	810	810	/	810	810	810
Guatteria parvifolia	AL1354	AL1354	AL1354	/	AL1354	AL1354
Guatteria pittieri	819	819	/	819	819	819
Guatteria pogonopus	807	0802	AL_715	0802	0807	0802
Guatteria pohliana	806	0806	RCF_3676	RCF_3676	0806	0806
Guatteria polyantha	890type	890type	/	890type	890type	890type
Guatteria polycarpa	891type	891type	/	891type	891type	891type
Guatteria pubens	805	805	/	805	805	805
Guatteria pudica	0249	0249	0249	0249	0249	0249
Guatteria punctata	692	0692	/	0692	0692	0692
Guatteria puncticulata	283	283	/	283	283	283
Guatteria ramiflora	951	951	/	951	951	951

Guatteria reflexa	Barros2735	Barros2735	Barros2735		Barros2735	
Guatteria rostrata	256	256	/	256	256	256
Guatteria rotundata	783	783	/	783	783	783
Guatteria rupestris	803	803	/	803	803	803
Guatteria scalarinervia	285	285	/	285	285	285
Guatteria scandens	183	183	/	183	183	183
Guatteria schomburgkiana	818	818	/	818	818	818
Guatteria scytophylla	559	559	/	559	559	559
Guatteria sellowiana	511	511	/	511	511	511
Guatteria sessilicarpa	1320	1320	/	1320	1320	1320
Guatteria sphaerantha	817	817	/	817	817	817
Guatteria subsessilis	0090	0090	/	0090	0090	0090
Guatteria talamancana	1319	1319	/	1319	1319	1319
Guatteria tenera	780	780	/	780	780	780
Guatteria tomentosa	815	815	/	815	815	815
Guatteria tonduzii	259	259	/	259	259	259
Guatteria ucayalina	549	549	/	549	549	549
Guatteria venezuelana	814	814	/	814	814	814
Guatteria verrucosa	882	882	/	882	882	882
Guatteria villosissima	564	564	/	564	564	564
Guatteria wachenheimii	689	689	/	689	689	689
Guatteria xylopioides	CHRISTO	CHRISTO	CHRISTO	/	/	/
Hexalobus crispiflorus	TC108	/	/	/	TC108	TC108
Hexalobus salicifolius	TC107	TC107	TC107	TC107	TC107	TC107
Hornschuchia citriodora	0106	/	/	/	0106	0106
Huberantha cerasoides	JB55	JB55	JB55	JB55	JB55	JB55
Huberantha henricii	TNWH52	/	TNWH52	TNWH52	/	TNWH52
Huberantha jenkinsii	TNW39	/	TNW39	TNW39	/	TNW39
Huberantha korinti	YVONNE	YVONNE	YVONNE	YVONNE	/	YVONNE
Huberantha nitidissima	TNW32	TNW32	TNW32	TNW32	/	TNW32
Huberantha pendula	MWC7069	MWC7069	MWC7069	MWC7069	MWC7069	MWC7069
Huberantha perrieri	TNWH77	/	TNWH77	TNWH77	/	TNWH77
Huberantha stuhlmannii	MWC7136	MWC7136	MWC7136	MWC7136	MWC7136	MWC7136
Huberantha tanganyikensis	TNW4445	/	TNW4445	TNW4445	/	TNW4445
Isolona campanulata	0531	0531	0531	0531	0531	0531
Isolona_capuronii_TC134	?	/	TC134	TC134	/	TC131
Isolona_cauliflora_TC46	?	/	TC46	TC46	/	TC46
Isolona_congolana_TC106	?	/	TC106	TC106	/	TC106
Isolona_cooperi_UDNA532	?	/	UDNA532	UDNA532	/	0532
Isolona_cooperi_	?	?	?	?	?	?
Isolona_dewevrei_TC111	?	/	TC111	TC111	/	TC111

Isolona_ghesquieri_TC121	?	/	TC121	TC121	/	TC121
Isolona_heinsenii_TC137	?	/	TC137	TC137	/	TC137
Isolona_hexaloba_TC54	?	/	TC54	TC54	/	TC54
Isolona_linearis_TC136	?	/	TC136	TC136	/	TC59
Isolona_maintlandii_TC59	?	/	TC59	TC59	/	TC136
Isolona_pleurocarpa_TC105	?	/	TC105	TC105	/	TC105
Isolona_stellata_TC31	?	/	TC31	TC31	/	TC31
Isolona_thonneri_TC134	?	/	TC134	TC134	/	TC134
Isolona_zenkeri_TC50	?	/	TC50	TC50	/	TC50
Isolona_zenkeri_TC52	?	/	TC52	TC52	/	TC52
Klarobelia inundata	0301	0301	0301	0301	0301	0301
Klarobelia megalocarpa	0486	/	/	/	0486	0486
Klarobelia stipitata	0253	0253	0253	0253	0253	0253
Letestudoxa bella	0145	0145	0145	0145	0145	0145
Letestudoxa glabrifolia	0164	0164	/	0164	0164	0164
Lettowianthus stellatus	TC55	TC55	/	TC55	TC55	TC55
Maasia discolor	JB114	JB114	JB114	JB114	JB114	JB114
Maasia glauca	JB17	/	/	/	JB17	JB17
Maasia sumatrana	JB22	JB22	JB22	JB22	JB22	JB22
Malmea dielsiana	0260	/	0260	0260	0260	0260
Malmea spnov	0197	0197	0197	0197	0197	0197
Malmea surinamensis	0850	/	/	/	0850	0850
Marsypopetalum littorale	JB13	JB13	JB13	JB13	JB13	JB13
Marsypopetalum modestum	JB71	JB71	JB71	JB71	JB71	JB71
Marsypopetalum pallidum	JB71	/	/	/	JB71	JB71
Meiocarpidium lepidotum	TNW123	TNW123	TNW123	TNW123	TNW123	TNW123
Meiogyne cylindrocarpa	JB94	/	/	/	JB94	JB94
Meiogyne_cylindrocarpa_TNW_92	TNW_92	TNW_92	TNW_92	TNW_92	/	TNW_92
Meiogyne flagelliflora	SPNOVTNW146	SPNOVTNW146	SPNOVTNW146	SPNOVTNW146	SPNOVTNW146	SPNOVTNW146
Meiogyne stenopetala	MWC7003	/	/	/	MWC7003	MWC7003
Meiogyne virgata	JB37	JB37	JB37	JB37	JB37	JB37
Melodorum fruticosum	JB	?	?	?	JB	JB
Mezzettia parviflora	JB32	/	/	/	JB32	JB32
Miliusa amplexicaulis	TNW141	/	TNW141	TNW141	/	TNW141
Miliusa balansae	TNW38	/	TNW38	TNW38	/	TNW38
Miliusa brahei	TNW26	/	TNW26	TNW26	/	TNW26
Miliusa butonensis	SPNOVTNWH5	/	SPNOVTNWH5	SPNOVTNWH5	/	SPNOVTNWH5
Miliusa campanulata	JB56	/	TNW_137	TNW_137	JB56	JB56

Miliusa cuneata	TNW142	/	TNW142	TNW142	/	TNW142
Miliusa dioeca	TNWH82	/	TNWH82	TNWH82	/	TNWH82
Miliusa fusca	TNW21	/	TNW21	TNW21	/	TNW21
Miliusa horsfieldii	JB4	/	TNW_144	TNW_144	JB4	JB4
Miliusa intermedia SPNOV	TNW30	/	TNW30	TNW30	/	TNW30
Miliusa koolsii	TNWH19	/	TNWH19	TNWH19	/	TNWH19
Miliusa lanceolata	TNWH18	/	TNWH18	TNWH18	/	TNWH18
Miliusa macropoda	TNWH2	/	TNWH2	TNWH2	/	TNWH2
Miliusa mollis	JB43	JB43	PK3207_JB99	PK3207_JB99	JB43	PK3207_JB99
Miliusa montana	TNWH98	/	TNWH98	TNWH98	TNWH98	TNWH98
Miliusa novoguineesis	TNWH80	/	TNWH80	TNWH80	/	TNWH80
Miliusa oropheoides SPNOV	TNW28	/	TNW28	TNW28	/	TNW28
Miliusa parviflora	TNW138	/	TNW138	TNW138	/	TNW138
Miliusa sclerocarpa	TNW23	/	TNW23	TNW23	/	TNW23
Miliusa sp	KHAO NAM PRAI TNW42	/	KHAO NAM PRAI TNW42	KHAO NAM PRAI TNW42	/	KHAO NAM PRAI TNW42
Miliusa sp	NAMNAOTNW143	/	NAMNAOTNW143	NAMNAOTNW143	/	NAMNAOTNW143
Miliusa thailandica spNOV	TNW51	/	TNW51	TNW51	/	TNW51
Miliusa thorelii	JB	/	JB	JB	/	JB
Miliusa traceyi	TNW27	/	TNW27	TNW27	/	TNW27
Miliusa umpangensis spNOV	TNW114	/	TNW114	TNW114	/	TNW114
Miliusa velutina	JB	JB	JB	JB	JB	JB
Mischogyne_congensi	?	/	/	MH286448	MH286457	MH286453
Mischogyne_elliotiana_var_sericea	?	/	/	MH286450	/	/
Mischogyne_elliotiana_var__elliotiana	?	/	/	MH286449	MH286458	MH286454
Mischogyne_gabonensis	?	/	/	MH286451	MH286459	MH286455
Mischogyne_iddi	?	/	/	MH286452	/	MH286456
Mischogyne_michelioides	?	/	/	EU169741	EU169764	EU169786
Mischogyne michelioides	1294	1294	1294	1294	1294	1294
Mitrella kentii	MWC7134	/	/	/	MWC7134	MWC7134
Mitrephora alba	JB62	JB62	JB62	JB62	JB62	JB62
Mitrephora macropoda	JB	JB	JB	JB	/	JB
Mitrephora polypyrena	JB41	/	/	/	JB41	JB41
Mitrephora teysmannii	JB79	/	/	/	JB79	JB79
Mkilua fragrans	1271	1271	1271	1271	1271	1271

Monanthotaxis ambrensis	?	?	?	/	?	?
Monanthotaxis angustifolia	?	?	?	/	?	?
Monanthotaxis atewae	?	?	?	/	?	?
Monanthotaxis barteri	?	?	?	/	?	?
Monanthotaxis boivinii	?	?	?	/	?	?
Monanthotaxis boivinii vbrevipedicellata	?	?	?	/	?	?
Monanthotaxis bokoli	?	?	?	/	?	?
Monanthotaxis breteler	?	?	?	/	?	?
Monanthotaxis buchananii	?	?	?	?	?	?
Monanthotaxis caesia	?	?	?	/	?	?
Monanthotaxis caffra	?	?	?	/	?	?
Monanthotaxis cauliflora	?	?	?	/	?	?
Monanthotaxis cf cauliflora	?	?	?	/	?	?
Monanthotaxis cf congoensis	?	?	?	?	?	?
Monanthotaxis_cf_dielsiana	?	?	?	/	?	?
Monanthotaxis cf glaucocarpa	?	?	?	/	?	?
Monanthotaxis cf glomerulata	?	?	?	/	?	?
Monanthotaxis chasei	?	?	?	/	?	?
Monanthotaxis congoensis	?	?	?	/	?	?
Monanthotaxis diclina	?	?	?	/	?	?
Monanthotaxis dictyoneura	?	?	?	/	?	?
Monanthotaxis discolor	?	?	?	/	?	?
Monanthotaxis discrepantinervia	?	?	?	/	?	?
Monanthotaxis elegans	?	?	?	/	?	?
Monanthotaxis enghiana	?	?	?	?	?	?_2
Monanthotaxis faulknerae	?	?	?	/	?	?
Monanthotaxis ferruginea	?	?	?	DSM089	?	?
Monanthotaxis filamentosa	?	?	DSM088	DSM088	?	?
Monanthotaxis foliosa	?	?	?	/	?	?
Monanthotaxis fornicata	?	?	?	?	?	?
Monanthotaxis geerling	?	?	?	/	?	?
Monanthotaxis gilletii	?	?	?	/	?	?
Monanthotaxis_glaucifolia	?	?	?	/	?	?

Monanthotaxis glomerulata	?	?	?	?	?	/
Monanthotaxis_hirsuta	?	?	?	/	?	?
Monanthotaxis heterantha	?	?	?	/	?	?
Monanthotaxis klainei vangustifolius	?	?	?	/	?	?
Monanthotaxis klainei vklainei	?	?	?	/	?	?
Monanthotaxis klainei vlastoursvillensis	?	?	?	/	?	?
Monanthotaxis laurentii	?	?	?	/	?	?
Monanthotaxis letestui	?	?	?	/	?	?
Monanthotaxis letouzeyi	?	?	?	/	?	?
Monanthotaxis littoralis	?	?	?	/	?	?
Monanthotaxis lucidula	?	?	?	/	?	?
Monanthotaxis mannii	?	?	?	/	?	?
Monanthotaxis maputensis	?	?	?	/	?	?
Monanthotaxis micrantha	?	?	?	/	?	?
Monanthotaxis nimbana	?	?	?	/	?	?
Monanthotaxis novmontana	?	?	?	/	?	?
Monanthotaxis obovata	?	?	?	?	?	?
Monanthotaxis orophila	?	?	?	/	?	?
Monanthotaxis parvifolia	?	?	?	/	?	?
Monanthotaxis parvifolia skenyensis	?	?	?	/	?	?
Monanthotaxis pellegrinii	?	?	?	/	?	?
Monanthotaxis poggei	?	?	?	/	?	?
Monanthotaxis pynaertii	?	?	?	/	?	?
Monanthotaxis_scamno petala	?	?	?	?	?	?
Monanthotaxis schweinfurthii	?	?	?	?	?	?
Monanthotaxis schweinfurthii vseretii	?	?	?	/	?	?
Monanthotaxis schweinfurthii vtisserantii	?	?	?	?	?	?
Monanthotaxis sororia	?	?	?	/	?	?
Monanthotaxis sp	?	/	/	/	?	?
Monanthotaxis_specnov	?	?	?	/	?	?
Monanthotaxis_specnov 2	?	?	?	/	?	?
Monanthotaxis trichantha	?	?	?	/	?	?

Monanthotaxis trichocarpa	?	?	?	?	?	?
Monanthotaxis valida	?	?	?	/	?	?
Monanthotaxis vogelii	?	?	?	/	?	?
Monanthotaxis whytei	0533	0533	0533	0533	0533	0533
Monanthotaxis wieringa	?	?	?	/	?	?
Monocarpia euneura	JB106	JB106	JB106	JB106	JB106	JB106
Monocarpia marginalis	TNW140	TNW140	TNW140	TNW140	TNW140	TNW140
Monocyclanthus vegnei	TC125	TC125	TC125	TC125	TC125	TC125
Monodora_angolensis_TC63	?	/	TC63	TC63	/	TC63
Monodora_brevipes_TC62	?	/	TC62	TC62	/	TC62
Monodora_carolinae_TC140	?	/	TC140	TC140	/	TC140
Monodora crispata	0534	/	/	0534	0534	0534
Monodora_globiflora_TC142	?	/	TC142	TC142	/	TC142
Monodora_grandidieri_TC123	?	/	TC123	TC123	/	TC123
Monodora_hastipetala_TC141	?	/	TC141	TC141	/	TC141
Monodora_junodii_TC139	?	/	TC139	TC139	/	TC139
Monodora_laurentii_TC122	?	/	TC122	TC122	/	TC122
Monodora_minor_143	?	/	TC143	TC143	/	TC143
Monodora myristica	0535	0535	0535	0535	0535	0535
Monodora_stenopetala_TC15	?	/	TC15	TC15	/	TC15
Monodora tenuifolia	0166	/	/	/	0166	0166
Monodora_tenuifolia_TC118	?	/	TC118	TC118	/	TC118
Monoon congestum	JB86	/	/	/	JB86	JB86
Monoon fuscum	JB	JB	JB	JB	JB	/
Monoon glabrum	JB7	/	/	/	JB7	JB7
Monoon_lateriflorum_JB15	?	/	/	/	JB15	JB15
Monoon longifolium	JB14	JB14	/	/	JB14	JB14
Monoon membranifolium	JB97	/	/	/	JB97	JB97
Monoon paradoxum	JB38	/	/	/	JB38	JB38
Monoon sclerophyllum	JB2	/	/	/	JB2	JB2
Monoon viridis	JB63	JB63	JB63	JB63	JB63	JB63
Monoon xanthopetalum	JB87	JB87	/	/	JB87	JB87
Mosannona costaricensis	0237	0237	0237	0237	0237	0237
Mosannona depressa	0489	0489	0489	0489	0489	0489

Mosannona depressa abscondita	1322	1322	1322	1322	1322	1322
Mosannona discolor	0186	0186	0186	0186	0186	0186
Mosannona garwoodii	0050	0050	0050	0050	0050	0050
Mosannona hypoglauca	1323	1323	1323	1323	1323	1323
Mosannona pacifica	0487	0487	0487	0487	0487	0487
Mosannona papillosa	0974	0974	0974	0974	0974	0974
Mosannona parva	1325	1325	/	1325	1325	1325
Mosannona raimondii	1324	1324	/	1324	1324	1324
Mosannona sp	0226	0226	/	0226	0226	0226
Mosannona vasquezii	0313	0313	0313	0313	0313	0313
Mosannona xanthochlora	?	/	/	/	/	?
Mwasumbia alba	TC85	TC85	TC85	TC85	TC85	TC85
Neo uvaria acuminatissima	JB101	JB101	/	/	JB101	JB101
Neo uvaria paralellivenia	JB39	JB39	JB39	JB39	JB39	JB39
Neo uvaria telopea SPNOV	TNW47	TNW47	TNW47	TNW47	TNW47	TNW47
Neostenanthera myristicifolia	0054	0054	0054	0054	0054	0054
Onychopetalum periquino	0017	0017	0017	0017	0017	0017
Ophrypetalum odoratum	TC65	TC65	TC65	TC65	TC65	TC65
Orophea celebica	JB25	JB25	/	/	JB25	JB25
Orophea creaghii	MWC7046	/	/	/	MWC7046	MWC7046
Orophea enterocarpa	JB53	JB	JB	JB	JB53	JB53
Orophea kerrii	JB60	JB60	JB60	JB60	JB60	JB60
Orophea polycarpa	JB73	JB73	/	/	JB73	JB73
Orophea_sp_0750_Mike	?	0750_Mike	/	/	0750_Mike	0750_Mike
OUT Degeneria vitiensis	?	?	?	/	?	?
OUT Eupomatia bennettii	1410	1410	1410	1410	1410	1410
OUT Galbulimima belgraveana	L12646	L12646	?	/	L12646	L12646
OUT Liriodendron chinense	0521	/	/	/	0521	0521
OUT Magnolia kobus	0520	0520	0520	/	0520	0520
OUT Persea americana	0484	0484	0484	0484	/	0484
Oxandra asbeckii	0629	/	/	/	0629	0629
Oxandra espintana	0268	0268	0268	0268	0268	0268
Oxandra euneura	0326	/	/	/	0326	0326
Oxandra longipetala	0254	/	/	/	0254	0254
Oxandra macrophylla	0300	0300	0300	0300	0300	0300
Oxandra polyantha	0308	/	/	/	0308	0308
Oxandra sphaerocarpa	0080	/	/	/	0080	0080
Oxandra venezuelana	0258	0258	0258	0258	0258	0258
Oxandra xylopioides	0280	/	/	/	0280	0280

Phaeanthus ebracteolatus	MWC7062	/	/	/	MWC7062	MWC7062
Phaeanthus sp LENDENS	JB	JB	JB	JB	JB	JB
Phaeanthus sp PNG	TNW12	TNW12	TNW12	TNW12	/	TNW12
Piptostigma fasciculatum	GENBANK	GENBANK	GENBANK	GENBANK	GENBANK	GENBANK
Piptostigma_macrophyllum	?	/	/	KU716125	KU716148	KU716135
Piptostigma mortehani	0505	0505	0505	0505	0505	0505
Piptostigma_multinervium	?	/	/	KU716126	KU716146	KU716133
Piptostigma_oyemense	?	/	/	KU716124	KU716147	KU716134
Piptostigma pilosum	0956	/	/	/	0956	0956
Piptostigma_submontanum	?	/	/	KU716123	KU716143	KU716130
Piptostima_macranthum	?	/	/	/	KU716145	KU716132
Platymitra macrocarpa	JB29	JB29	JB29	JB29	JB29	JB29
Platymitra sp TH	TNW115	TNW115	TNW115	TNW115	/	TNW115
Polyalthia borneensis	JB90	/	/	/	JB90	JB90
Polyalthia bullata	TNW18	TNW18	TNW18	TNW18	/	TNW18
Polyalthia cauliflora	JB28	/	/	/	JB28	JB28
Polyalthia_cerasoides	?	AY518827	/	/	AY319016	AY319130
Polyalthia_cf__glabra	?	AY319022	/	/	AY518824	AY319136
Polyalthia_cf__longifolia	?	?	/	/	?	?
Polyalthia celebica	JB16	/	/	/	JB16	JB16
Polyalthia_coffeoides	?	AY518828	/	/	AY319018	AY319132
Polyalthia_congesta	?	EU522233	/	/	EU522288	EU522178
Polyalthia cinnamomea	JB84	/	/	/	JB16	JB16
Polyalthia debilis	JB75	/	/	/	JB75	JB75
Polyalthia flagellaris	MWC7066	/	/	/	MWC7066	MWC7066
Polyalthia_korinti	?	AY518782	/	/	AY319032	AY319146
Polyalthia_longifolia	?	AY518781	/	/	AY319024	AY319138
Polyalthia_longipes_JB91	?	AY518785	/	/	AY319025	AY319139
Polyalthia longirostris	JB	/	/	/	JB	JB
Polyalthia obliqua	MWC7064	/	/	/	MWC7064	MWC7064
Polyalthia_pendula	?	AY518822	/	/	AY319029	AY319143
Polyalthia_rumphii	?	AY518852	/	/	AY319030	AY319144

Polyalthia_sclerophylla	?	AY518791	/	/	AY319031	AY319145
Polyalthia stenopetala	JB59	/	/	/	JB59	JB59
Polyalthia_stuhlmannii	?	/	/	/	AY319034	AY319148
Polyalthia subcordata	IIJB	/	/	/	IIJB	IIJB
Polyalthia suberosa	0536	0536	0536	0536	0536	0536
Polyalthia_viridis	?	AY518833	/	/	AY319038	AY319152
Polyalthia_xanthopetala	?	AY518784	/	/	AY319040	AY319154
Polyceratocarpus microtrichus	YB3	YB3	YB3	YB3	/	/
Polyceratocarpus_parviflorus	?	/	/	?	?	/
Polyceratocarpus pellegrini	YB4	YB4	YB4	YB4	/	/
Polyceratocarpus sp	YB2	YB2	YB2	YB2	YB2	YB2
Popowia hirta	JB24	JB24	JB24	JB24	JB24	JB24
Popowia odoardi	JB96	/	/	/	JB96	JB96
Popowia pisocarpa	JB26	JB	JB	JB	JB26	JB26
Porcelia steinbachii	0537	/	/	/	0537	0537
Pseudartabotrys letestui	0481	0481	0481	0481	0481	0481
Pseudephedranthus enigmaticus	Guianas	Guianas	Guianas	Guianas	Guianas	Guianas
Pseudephedranthus fragrans	0033		0033	0033	0033	0033
Pseudephedranthus sp	TNWH139	TNWH139	TNWH139	TNWH139	TNWH139	TNWH139
Pseudomalmea diclina	0305	0305	0305	0305	0305	0305
Pseudomalmea sp	0768	/	/	/	0768	0768
Pseudoxandra lucida	0307	0307	0307	0307	0307	0307
Pseudoxandra polyphleba	0081	0081	0081	0081	0081	0081
Pseudoxandra spiritussancti	0110	0110	0110	0110	0110	0110
Pseuduvaria coriacea	JB	/	/	/	JB	JB
Pseuduvaria fragrans	YVONNE	YVONNE	YVONNE	YVONNE	YVONNE	YVONNE
Pseuduvaria megalopus	JB	/	/	/	JB	JB
Pseuduvaria pamattonis	JB105	JB105	/	/	JB105	JB105
Pseuduvaria phuyensis	JB	/	/	/	JB	JB
Pseuduvaria rugosa	JB69	JB69	/	/	JB69	JB69
Pseuduvaria setosa	TNW135	TNW135	TNW135	TNW135	/	TNW135
Pyramidanthe prismatica	?	?	/	?	?	/
Ruizodendron ovale	0088	0088	0088	0088	0088	0088
Sageraea hookeri	TNW2	TNW2	TNW2	TNW2	/	TNW2
Sageraea lanceolata	JB89	JB89	JB89	JB89	JB89	JB89
Sageraea macropoda spNOV	TNW127	TNW127	TNW127	TNW127	/	TNW127

Sageraea sp	BORNEOTNW8	BORNEOTNW8	BORNEOTNW8	BORNEOTNW8	/	BORNEOTNW8
Sanrafaelia rufonammari	TC109	TC109	TC109	TC109	TC109	TC109
Sapranthus microcarpus	JBPM8457	/	/	/	JBPM8457	JBPM8457
Sapranthus viridiflorus	0211	0211	0211	0211	0211	0211
Siamocananga GEN NOV	TNW94	TNW94	TNW94	TNW94	TNW94	TNW94
Siamocananga	TNW147	TNW147	TNW147	TNW147	/	TNW147
Sirdavidia_solanonna	?	/	/	/	?	?
Sphaerocoryne gracilis	2	?_2	?	?	?_2	?_2
Stelechocarpus burahol	JB	JB	JB	JB	JB	JB
Stelechocarpus cauliflorus	JB1	JB1	JB1	JB1	JB1	JB1
Stelechocarpus expansus spNOV	TNW49	TNW49	TNW49	TNW49	/	TNW49
Stenanona costaricensis	0222	0222	0222	0222	0222	0222
Stenanona panamensis	0243	0243	/	/	0243	0243
Tetrameranthus duckei	0028	/	/	/	0028	0028
Tetrameranthus laomae	0030	/	/	/	0030	0030
Toussaintia orientalis	TC130	TC130	TC130	TC130	/	TC130
Tridimeris sp	JB	/	/	/	JB	JB
Tridimeris sp	TNWH109	TNWH109	TNWH109	TNWH109	TNWH109	TNWH109
Trigynaea duckei	0264	/	/	/	0264	0264
Trigynaea lanceipetala	0318	0318	0318	0318	0318	0318
Trivalvaria macrophylla	MR184	/	BORNEO_FR_TNW_H128	BORNEO_FR_TNW_H128	MR184	BORNEO_FR_TNW_H128
Trivalvaria mollis spNOV	TNW1788	TNW1788	TNW1788	TNW1788	TNW1788	TNW1788
Trivalvaria pumila	TNWH42	/	TNWH42	TNWH42	/	TNWH42
Trivalvaria sp PHANGNGA	TNW41	/	TNW41	TNW41	/	TNW41
Trivalvaria sp SAKAOPRAI	TNW145	/	TNW145	TNW145	/	TNW145
Trivalvaria sp SAOBANGKRAM	TNW40	/	TNW40	TNW40	/	TNW40
Trivalvaria sp SAOSOIDAO	TNW16	/	TNW16	TNW16	/	TNW16
Trivalvaria sp UMPANG	TNW116	/	TNW116	TNW116	/	TNW116
Unonopsis asterantha	1142	1142	/	1142	1142	1142
Unonopsis bullata	1343	1343	/	1343	1343	1343
Unonopsis elegantissima	0327	0327	/	0327	0327	0327
Unonopsis floribunda	0079	0079	/	0079	0079	0079
Unonopsis glaucopetala	0191	0191	/	0191	0191	0191
Unonopsis guatterioides	0085	0085	/	0085	0085	0085
Unonopsis hammelii	1342	1342	/	1342	1342	1342
Unonopsis osae	1341	1341	/	1341	1341	1341
Unonopsis penduliflora	0086	0086	/	0086	0086	0086
Unonopsis perrottetii	0971	0971	/	0971	0971	0971

Unonopsis peruviana	1167	1167	/	1167	1167	1167
Unonopsis pittieri	0223	0223	/	0223	0223	0223
Unonopsis rufescens	714	0705	/	0705	0714	0714
Unonopsis sanctae teresae	0108	0108	/	0108	0108	0108
Unonopsis spectabilis	1181	1181	/	1181	1181	1181
Unonopsis stipitata	0329	0329	0329	0329	0329	0329
Unonopsis storkii	1344	1344	/	1344	1344	1344
Unonopsis theobromifolia	0200	0200	/	0200	0200	0200
Unonopsis veneficiorum	0497	0497	/	0497	0497	0497
Uvaria acuminata	?	?	/	?	?	/
Uvaria afzelii	?	?	/	?	?	/
Uvaria angolensis	?	?	/	?	?	/
Uvaria anonoides	?	?	/	?	?	/
Uvaria antsiranensis	?	?	/	?	?	/
Uvaria argenteum	?	?	/	?	?	/
Uvaria baumannii	?	?	/	?	?	/
Uvaria boniana	?	?	/	?	?	/
Uvaria borneensis	?	?	/	?	?	?
Uvaria buchholzii	?	?	/	?	?	/
Uvaria calamistrata	?	?	/	?	?	/
Uvaria chamae	172	?	/	?	0172	0172
Uvaria_cherrevensis		?	/	?	?	?
Uvaria clavata	?	?	/	?	?	/
Uvaria clementis	1000	?	/	?	1000	1000
Uvaria commersoniana	?	?	/	?	?	/
Uvaria concava	?	?	/	?	?	/
Uvaria cordata	?	?	?	?	?	?
Uvaria_cuneifolia	?	?	/	?	?	?
Uvaria dasoclema	?	?	/	?	?	/
Uvaria dependens	?	?	/	?	?	/
Uvaria dulcis	?	?	/	?	?	?
Uvaria elmeri	?	?	/	?	?	/
Uvaria excelsa	?	?	/	?	?	/
Uvaria faulknerae	?	?	/	?	?	/
Uvaria flava	?	?	/	?	?	/
Uvaria glabra	?	?	/	?	?	/
Uvaria grandiflora	1026	1026	/	1026	1026	1026
Uvaria griffithii	999	?	/	?	0999	0999
Uvaria hahnii	?	?	/	?	?	/
Uvaria hamiltonii	?	?	/	?	?	/
Uvaria hirsuta	?	?	/	?	?	/
Uvaria laha	?	?	/	?	?	/
Uvaria leichhardtii	?	?	/	?	?	/
Uvaria leptocladon	?	?	/	?	?	/
Uvaria lobbiana	?	?	/	?	?	/
Uvaria lucida	0538	0538	0538	0538	0538	0538
Uvaria lurida	?	?	/	?	?	/
Uvaria macropoda	?	?	/	?	?	/

Uvaria manjensis	?	?	/	?	?	/
Uvaria marenteria	?	?	?	?	?	?
Uvaria micrantha	?	?	/	?	?	/
Uvaria pandensis	?	?	/	?	?	/
Uvaria pauciovulata	?	?	/	?	?	/
Uvaria rosenbergiana	?	/	/	/	?	/
Uvaria rufa	?	?	/	?	?	/
Uvaria rupestris	?	?	/	?	?	/
Uvaria sambiranensis	?	?	/	?	?	/
Uvaria sankowskyi	?	?	/	?	/	/
Uvaria scabrida	?	?	?	/	?	?
Uvaria scabridula	?	?	/	?	?	/
Uvaria semecarpifolia	?	?	/	?	?	/
Uvaria siamensis	Lillian	Lillian	/	Lillian	Lillian	Lillian
Uvaria sphenocarpa	?	?	/	?	?	/
Uvaria topazensis	?	?	/	?	?	/
Uvaria uhrii	?	?	/	?	?	/
Uvaria unguiculata	?	?	/	?	?	/
Uvaria versicolor	?	?	/	?	?	/
Uvaria welwitschii	?	?	/	?	?	/
Uvaria wrayi	?	?	/	?	?	/
Uvaria zeylanica	?	?	/	?	?	/
Uvariastrum insculptum	1295	1295	1295	1295	1295	1295
Uvariastrum pynaertii	1298	/	/	/	1298	1298
Uvariodendron kirkii	TC83	/	/	/	TC83	TC83
Uvariodendron molundense	TC84	TC84	TC84	TC84	TC84	TC84
Uvariopsis korupensis	TC86	/	/	/	TC86	TC86
Uvariopsis spGabon	TC88	TC88	TC88	TC88	TC88	TC88
Uvariopsis tripetala	TC132	/	/	/	TC132	TC132
Xylopia humblotiana	J24	/	/	J24	/	/
Xylopia aethiopica	X1	X1	/	X1	/	X1
Xylopia ambanjensis	?	/	?	J1	/	J1
Xylopia aromatica	X76	X76	/	X76	/	X76
Xylopia aurantiiodora	X9	/	/	X9	/	X9
Xylopia bakoensis	spnov	X6	?	X6	/	X6
Xylopia bemarivensis	X11	/	/	X11	/	X11
Xylopia benthamii	X80	X80	/	X80	/	X80
Xylopia buxifolia	X31	X31	/	X31	/	X31
Xylopia calophylla	X35	X35	/	/	/	/
Xylopia cf fananehanensis	X20	X20	/	X20	/	X20
Xylopia cf lemurica	J13	/	/	J13	/	J13
Xylopia elliptica	X84	X84	/	/	/	/
Xylopia ferruginea	1010	1010	1010	1010	1010	1010
Xylopia flamignii	X22	/	/	X22	/	X22
Xylopia frutescens	0248	/	/	/	0248	0248
Xylopia frutescens varferr	X69	X69	/	X69	/	X69

Xylopia frutescens varfrut	X24	X24	/	X24	/	X24
Xylopia hypolampra	119	X39	/	X39	0119	0119
Xylopia kalabenonensis	X21	X21	/	X21	/	X21
Xylopia lamarckii	J3	/	/	J3	/	/
Xylopia lastelliana	J27	/	/	J27	/	J27
Xylopia letestui	X29	X29	/	X29	/	X29
Xylopia longipetala	X98	/	/	X98	/	X98
Xylopia lukei spnov	J7	/	/	J7	/	J7
Xylopia madagascariensis	J25	/	/	J25	/	/
Xylopia malayana	?	X3	?	X3	/	X3
Xylopia mildbraedii	X97	/	/	X97	/	X97
Xylopia mini buxifolia	X32	X32	/	X32	/	X32
Xylopia mwasumbii	?	X2	?	X2	/	X2
Xylopia nitida	X81	X81	/	X81	/	X81
Xylopia ntsua spnov	X19	X19	/	X19	/	X19
Xylopia pallescens	X8	/	/	X8	/	X8
Xylopia parviflora	X33	X37	/	X33	/	X33
Xylopia perrieri	J10	/	/	J10	/	J10
Xylopia peruviana	0539	0539	0539	0539	0539	0539
Xylopia pierrei	?	X4	?	X4	/	X4
Xylopia polyantha	X12	/	/	X12	/	X12
Xylopia pulchella	?	?	?	?	/	?
Xylopia quintasii	X27	X7	?	X7	/	X7
Xylopia sahafariensis	?	/	?	J5	/	J5
Xylopia sclerophylla spnov	J26	/	/	J26	/	J26
Xylopia sp	J11	/	/	J11	/	J11
Xylopia staudtii	X30	X30	/	X30	/	X30
Xylopia vielana	?	X5	?	X5	/	X5

Table 1. Table of 819 species of the Annonaceae with accession numbers. ‘/’ indicates that the species was not sampled for that specific marker. ‘?’ indicates that we did not know the accession number of the species.

B. Script: Evolutionary rates

```
#Packages
library(ape)

library(phytools)

library(phangorn)

library(adephylo)

library(ggplot2)

library(nlme)

#Reading data
phylo <- read.nexus("C:/Users/silke/Desktop/unief bestanden/2e master/Thesis/FINAL ANALYSES/tree_likelihood.tre")
chrono <- read.tree("C:/Users/silke/Desktop/unief bestanden/2e master/Thesis/FINAL ANALYSES/tree_bayesian_HDI.tre")

#Data exploration
tree <- phylo

#

tree$edge.length <- phylo$edge.length/chron$edge.length

#

Plot the trees

#

Phylogram with clades highlighted

#

plotTree(tree = phylo, type ='fan', lwd=1, fsize = 0.04, lend =1, color = "black")
arc.cladelabels(node = 828, text = 'long branch clade', orientation = 'curved', col = "blue", cex = 1, ln.offset = 1.02, lab.offset = 1.06)
arc.cladelabels(node = 1357, text = 'short branch clade', orientation = 'curved', col = "red", cex = 1, ln.offset = 0.74, lab.offset = 0.78)

plotTree(tree = chrono, type ='fan', lwd=1, fsize = 0.04, lend =1, color = "black")
arc.cladelabels(node = 828, text = 'long branch clade', orientation = 'curved', col = "blue", cex = 1, ln.offset = 1.2, lab.offset = 1.2)
arc.cladelabels(node = 1357, text = 'short branch clade', orientation = 'curved', col = "red", cex = 1, ln.offset = 1.1, lab.offset = 1.1)

plotTree(tree = tree, type ='fan', lwd=0.4, fsize = 0.04, lend =1, color = "black")
arc.cladelabels(node = 828, text = 'long branch clade', orientation = 'curved', col = "blue", cex = 1, ln.offset = 1.02, lab.offset = 1.06)
arc.cladelabels(node = 1357, text = 'short branch clade', orientation = 'curved', col = "red", cex = 1, ln.offset = 0.7, lab.offset = 0.7)
```

```
Phylogram with clades painted - path length

phylo_colors <- paintSubTree(phylo,node=1357,state="2")

#

phylo_colors <- paintSubTree(phylo_colors,node=828,state="3")

#

cols<-c("black","red","blue"); names(cols)<-1:3

#

plotSimmap(phylo_colors,colors = cols, pts=F, type="fan",lwd=0.4,fsize= 0.08, tips=0:(Ntip(phylo_colors)-1), part=1-1/Ntip(phylo_colors), lend=1)

#

Phylogram with tips painted - species branch lengths

#

phylo_colors2 <- paintBranches(phylo, edge = clade.members(1357, phylo, tip.labels=F),  state="2")

#

phylo_colors2 <- paintBranches(phylo_colors2, edge = clade.members(828, phylo, tip.labels=F), state = "3")

#

cols<-c("black","red","blue"); names(cols)<-1:3

#

plotSimmap(phylo_colors2,colors = cols, pts=F, type="fan",lwd=0.4,fsize= 0.08, tips=0:(Ntip(phylo_colors2)-1), part=1-1/Ntip(phylo_colors2), lend=1)

#Analysing the data
For tree (substitutions/site/my)


malme <-extract.clade(phy = tree,node = 1357)
anna <- extract.clade(phy = tree,node = 828)


Calculating the path length


malme_path <- distRoot(malme)

anna_path <- distRoot(anna)

df2 <- data.frame(c(unname(malme_path), unname(anna_path)), c(rep("short", length(malme_path)), rep("long", length(anna_path))))
names(df2) <- c("branches", "clades")
```

```r
ggplot(df2, aes(x=clades, y=branches)) +
    geom_boxplot(varwidth = TRUE, alpha=0.7, fill = c("blue", "red")) +
    theme(legend.position="none") +
    labs(x=" ", y = "substitutions/site/my")+
    #ggtitle("path length")+
    theme_classic()

wilcox.test(malme_path, anna_path, alternative = 'less')

min <- min(c(annon_path, malme_path))*1000000

max <- max(c(annon_path, malme_path))*1000000

ax <- pretty(min:max, n = 50)/1000000

hist(annon_path, breaks = ax, xlim = c(0, 0.03), ylim = c(0, 40), col = scales::alpha("blue", .5), border = F, main = NULL, cex.lab = 1,  xlab = "Rate")

hist(malme_path, breaks = ax, xlim = xlim, col = scales::alpha("red", .5), border = F, add = T)

Calculating the species branch length


malme_species <- malme$edge.length[malme$edge[,2]<= Ntip(malme)]
anna_species <- anna$edge.length[anna$edge[,2]<= Ntip(anna)]

df <- data.frame(c(unname(malme_species), unname(anna_species)), c(rep("short", length(malme_species)), rep("long", length(anna_species))))
names(df) <- c("branches", "clades")


ggplot(df, aes(x=clades, y=branches)) +
    geom_boxplot(varwidth = TRUE, alpha=0.7, fill = c("blue", "red")) +
    theme(legend.position="none") +
    labs(x=" ", y = "substitutions/site/my")+
    #ggtitle("path length")+
    theme_classic()

wilcox.test(malme_species, anna_species, alternative = 'less')

# For a 100 trees

annon_path_red <- list()
for(i in 1:100) annon_path_red[[i]] <- distRoot(result[[i]])

wilcox_tests <- list()
for(i in 1:100) wilcox_tests[[i]] <-  wilcox.test(malme_path, annon_path_red[[i]], "less")
p_values <- vector()
for(i in 1:100) p_values[[i]] <- wilcox_tests[[i]]$p.value

df3 <- data.frame(c(unname(malme_path), unname(annon_path_red[[63]])), c(rep("short", length(malme_path)), rep("long", length(annon_path_red[[63]]))))
names(df3) <- c("branches", "clades")
```

```r
ggplot(df3, aes(x=clades, y=branches)) +
    geom_boxplot(varwidth = TRUE, alpha=0.7, fill = c("blue", "red")) +
    theme(legend.position="none") +
    labs(x=" ", y = "substitutions/site/my")+
    #ggtitle("path length")+
    theme_classic()

min <- min(c(annon_path_red[[63]], malme_path))*1000000

max <- max(c(annon_path_red[[63]], malme_path))*1000000

ax <- pretty(min:max, n = 50)/1000000

hist(annon_path_red[[63]], breaks = ax, xlim = c(0, 0.03), ylim = c(0, 30), col = scales::alpha("blue", .5), border = F, main = NULL, cex.lab = 1,  xlab = "Substitutions/site/my")

hist(malme_path, breaks = ax, xlim = xlim, col = scales::alpha("red", .5), border = F, add = T)

annon_tips_red <- list()
for(i in 1:100) annon_tips_red[[i]] <- result[[i]]$edge.length[result[[i]]$edge[,2] <= Ntip(result[[i]])]
wilcox_tests <- list()
for(i in 1:100) wilcox_tests[[i]] <-  wilcox.test(malme_path, annon_path_red[[i]], "less")
p_values <- vector()
for(i in 1:100) p_values[[i]] <- wilcox_tests[[i]]$p.value

df4 <- data.frame(c(unname(malme_species), unname(annon_tips_red[[91]])), c(rep("short", length(malme_species)), rep("long", length(annon_tips_red[[91]]))))
names(df4) <- c("branches", "clades")


ggplot(df4, aes(x=clades, y=branches)) +
    geom_boxplot(varwidth = TRUE, alpha=0.7, fill = c("blue", "red")) +
    theme(legend.position="none") +
    labs(x=" ", y = "substitutions/site/my")+
    #ggtitle("path length")+
    theme_classic()

min <- min(c(annon_tips_red[[91]], malme_tips))*10000000

max <- max(c(annon_tips_red[[91]], malme_tips))*10000000

ax <- pretty(min:max, n = 50)/10000000

hist(annon_tips_red[[91]], breaks = ax, xlim = c(0, 0.0035), ylim = c(0, 70), col = scales::alpha("blue", .5), border = F, main = NULL, cex.lab = 1, xlab = "Substitutions/site/my")

hist(malme_tips, breaks = ax, xlim = xlim, col = scales::alpha("red", .5), border = F, add = T)
```

C. Table mortality data

X	Family	Genus	Species	Mortality rate	Subfamily
1	Annonaceae	Alphonsea	Alphonsea_javanica	1.04738028	Malmeoideae
2	Annonaceae	Anaxagorea	Anaxagorea_dolichocarpa	3.385456144	Anaxagoreoideae
3	Annonaceae	Annickia	Annickia_chlorantha_0976	0.726397979	Malmeoideae
4	Annonaceae	Annickia	Annickia_polycarpa	1.980708327	Malmeoideae
5	Annonaceae	Anonidium	Anonidium_mannii	0.651058576	Annonoideae
6	Annonaceae	Bocageopsis	Bocageopsis_multiflora_0965	0.830267206	Malmeoideae
7	Annonaceae	Cleistopholis	Cleistopholis_patens	3.757479389	Ambavioideae
8	Annonaceae	Duguetia	Duguetia_echinophora_0372	0.917281928	Annonoideae
9	Annonaceae	Duguetia	Duguetia_manausensis	0.657123987	Annonoideae
10	Annonaceae	Duguetia	Duguetia_spixiana_0281_0734	1.994295485	Annonoideae
11	Annonaceae	Duguetia	Duguetia_staudtii_0854	2.013227329	Annonoideae
12	Annonaceae	Duguetia	Duguetia_surinamensis_0455	0.818780314	Annonoideae
13	Annonaceae	Fusaea	Fusaea_longifolia_0634	1.786236658	Annonoideae
14	Annonaceae	Greenwayodendron	Greenwayodendron_suaveolens_MWC7037	0.446415241	Malmeoideae
16	Annonaceae	Guatteria	Guatteria_punctata_692	3.4331121196	Annonoideae
17	Annonaceae	Hexalobus	Hexalobus_crispiflorus_TC108	2.276299469	Annonoideae
18	Annonaceae	Isolona	Isolona_thonneri_TC134	1.410305389	Annonoideae
19	Annonaceae	Lettowianthus	Lettowianthus_stellatus_TC55	1.371607549	Ambavioideae
20	Annonaceae	Malmea	Pseudomalmea_diclina_0305	1.097672451	Malmeoideae
21	Annonaceae	Monodora	Monodora_myristica_0535	2.629979594	Annonoideae
22	Annonaceae	Monodora	Monodora_tenuifolia_TC118	1.809538143	Annonoideae
23	Annonaceae	Oxandra	Oxandra mediocris	3.172441663	Malmeoideae
24	Annonaceae	Oxandra	Oxandra_asbeckii_0629	0.859828991	Malmeoideae
25	Annonaceae	Oxandra	Oxandra_riedeliana	2.000916302	Malmeoideae
26	Annonaceae	Oxandra	Oxandra_xylopioides_0280	1.226275845	Malmeoideae
27	Annonaceae	Polyalthia	Greenwayodendron_oliveri_0958	2.110340329	Malmeoideae
29	Annonaceae	Polyalthia	Monoon sumatrana	2.276842686	Malmeoideae
30	Annonaceae	Popowia	Bocagea pisocarpa	1.586879356	Malmeoideae
31	Annonaceae	Pseudoxandra	Pseudoxandra_cuspidata	2.409922223	Malmeoideae
32	Annonaceae	Ruizodendron	Ruizodendron_ovale_0088	1.869097587	Malmeoideae
33	Annonaceae	Unonopsis	Unonopsis_duckei	1.039789068	Malmeoideae
34	Annonaceae	Unonopsis	Unonopsis_floribunda_0079	1.905233515	Malmeoideae
35	Annonaceae	Unonopsis	Unonopsis_guatterioides_0085	2.292953019	Malmeoideae
36	Annonaceae	Unonopsis	Unonopsis_rufescens_0714	0.617258687	Malmeoideae
37	Annonaceae	Xylopia	Xylopia_aethiopica_X1	2.213662504	Annonoideae
38	Annonaceae	Xylopia	Xylopia_amazonica	2.66400824	Annonoideae
39	Annonaceae	Xylopia	Xylopia_chrysophylla	1.055752393	Annonoideae
40	Annonaceae	Xylopia	Xylopia_ferruginea_1010	1.146080119	Annonoideae
41	Annonaceae	Xylopia	Xylopia_hypolampra_0119	1.193771593	Annonoideae
42	Annonaceae	Xylopia	Xylopia_nitida_X81	1.206769203	Annonoideae
43	Annonaceae	Xylopia	Xylopia_parviflora_X33	1.919650987	Annonoideae
44	Annonaceae	Xylopia	Xylopia_quintasii_X27	1.5108061	Annonoideae
45	Annonaceae	Xylopia	Xylopia_sericea	1.26306687	Annonoideae
46	Annonaceae	Xylopia	Xylopia_staudtii_X30	2.917886189	Annonoideae
47	Annonaceae	Polyalthia	Polyalthia_flagellaris_MWC7066	1.55224944	Malmeoideae

Table 2. Table of the mortality data of 47 species. However, only 31 species were also included in our phylogenetic tree and used to evaluate the correlation between generation time and substitution rate.

D. Script: Mortality rates

```r
# Mortality rates
#
library(phytools)

library(adephylo)

library(ggplot2)

library(nlme)
library(phangorn)

library(caper)

#
Read the data
#
phylo <- read.nexus("C:/Users/silke/Desktop/unief bestanden/2e master/Thesis/FINAL ANALYSES/tree_likelihood.tre")
#
chron <- read.tree("C:/Users/silke/Desktop/unief bestanden/2e master/Thesis/FINAL ANALYSES/tree_bayesian_HDI.tre")
#
tree <- phylo
#
tree$edge.length <- phylo$edge.length/chron$edge.length
#
table <- read.table("C:/Users/silke/Desktop/unief bestanden/2e master/Thesis/FINAL ANALYSES/Mortality data/Ann_species_mort_updated.csv", sep = ";", header = TRUE)
#
tree_red <- keep.tip(tree, tree$tip.label[which(tree$tip.label %in% table[[7]])])
#
tree_red <- drop.tip(phy = tree_red, "Lettowianthus_stellatus_TC55")
#
malme_red <- extract.clade(tree_red, 50)
#
annon_red <- extract.clade(tree_red, 33)
#

#
Path lengths
#
malme_red_path <- distRoot(malme_red) ; names(malme_red_path) <- malme_red$tip.label

#
annon_red_path <- distRoot(annon_red) ; names(annon_red_path) <- annon_red$tip.label

#
all_data_path <- c(malme_red_path, annon_red_path)
#
table_reduced <- table[table[[7]] %in% names(all_data_path),]
```

```
#
table_reduced <- table_reduced[c(7,8)]
#
data <- table_reduced[order(table_reduced[1]),]
#

Species branch lengths
#


malme_red_tips <- malme_red$edge.length[malme_red$edge[,2] <= Ntip(malme_red)]
#
names(malme_red_tips) <- malme_red$tip.label[malme_red$edge[,2][malme_red$edge[,2] <= Ntip(malme_red)]]
#
annon_red_tips <- annon_red$edge.length[annon_red$edge[,2] <= Ntip(annon_red)]
#
names(annon_red_tips) <- annon_red$tip.label[annon_red$edge[,2][annon_red$edge[,2] <= Ntip(annon_red)]]
#
all_data_tips <- c(malme_red_tips, annon_red_tips)
#
# Data object

#
data[3] <- all_data_path[order(names(all_data_path))]
#
data[4] <- all_data_tips[order(names(all_data_tips))]
#
data[[5]] <- 1/data[[2]]
#
names(data) <- c("FullSpeciesName", "ann.mort.rate", "path", "tips", "generation")
#
# pgls path ~ generation
#
pglsModel <- gls(path ~ generation, correlation = corBrownian(phy = tree_red), data = data, method = "ML")

#
summary(pglsModel)

#
plot(data$generation, data$path, xlab="Generation time", ylab="Path branch length")
#
abline(a = coef(pglsModel)[1], b = coef(pglsModel)[2], lty = 1)

#
# pgls path ~ ann.mort.rate
#
pglsModel <- gls(path ~ ann.mort.rate, correlation = corBrownian(phy = tree_red), data = data, method = "ML")
```

```
#
summary(pglsModel)

#
plot(data$ann.mort.rate, data$path, xlab="Mortality rate", ylab="Path branc
h length")
#
abline(a = coef(pglsModel)[1], b = coef(pglsModel)[2], lty = 1)

#
# pgls tips ~ generation
#
pglsModel <- gls(tips ~ generation, correlation = corBrownian(phy = tree_re
d), data = data, method = "ML")

#
summary(pglsModel)

#
plot(data$generation, data$tips, xlab="Generation time", ylab="Species bran
ch length")
#
abline(a = coef(pglsModel)[1], b = coef(pglsModel)[2], lty = 1)

#
# pgls tips ~ ann.mort.rate
#
pglsModel <- gls(tips ~ ann.mort.rate, correlation = corBrownian(phy = tree
_red), data = data, method = "ML")

#
summary(pglsModel)

#
plot(data$ann.mort.rate, data$tips, xlab="Mortality rate", ylab="Species br
anch length")
#
abline(a = coef(pglsModel)[1], b = coef(pglsModel)[2], lty = 1)

#
```