Appendix S3: Statistical Analyses for Kitson *et al.* 2016
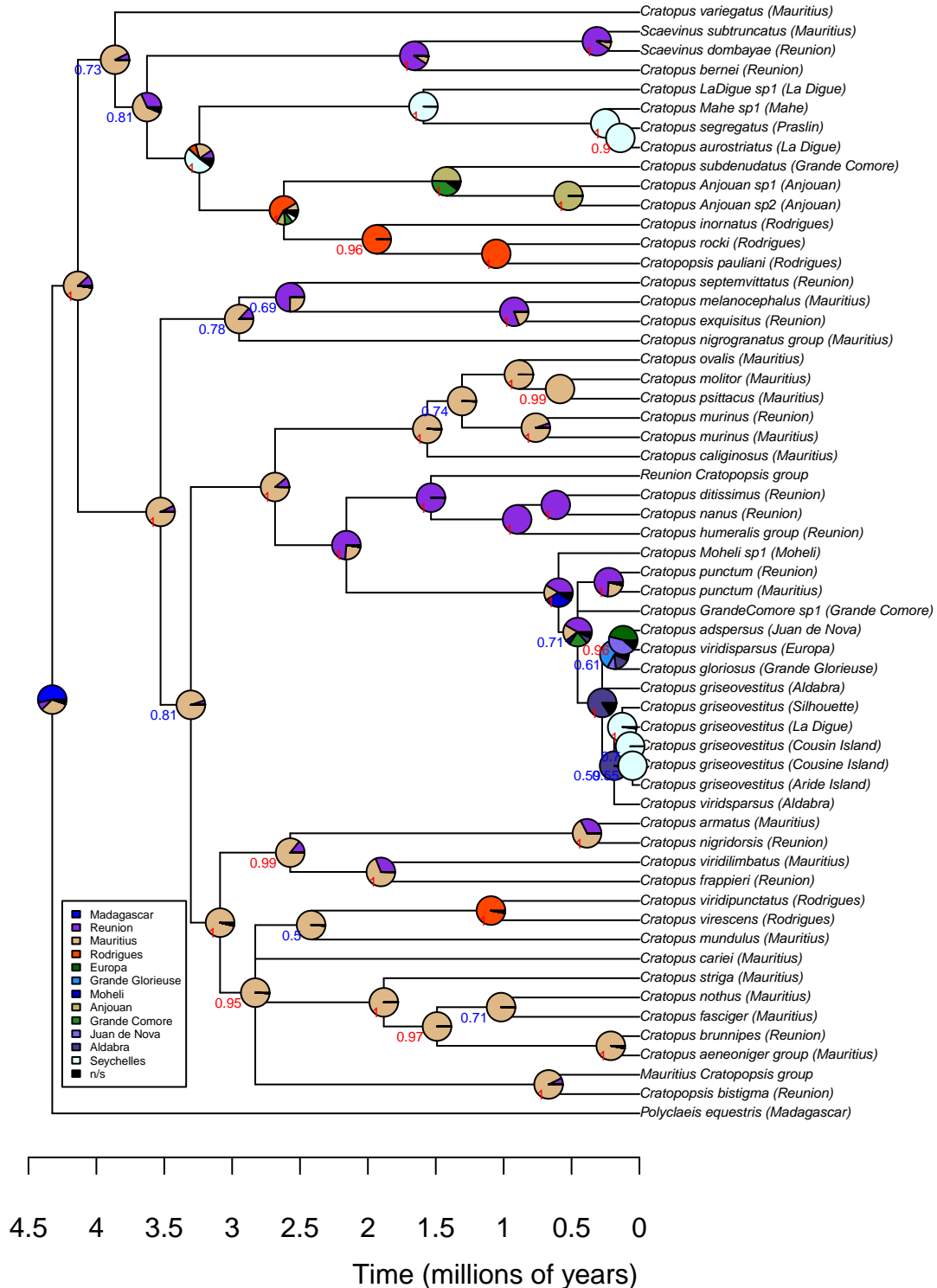
Introduction

The purpose of this appendix is to allow users to follow our statistical analyses for both the plotting of biogeographic analysis results and the subsequent statistics performed on node ages. Analyses are performed using separate R scripts called by this document and users should refer to the unrendered Cratopus_statistical_SI.Rmd file for links to the relevant scripts.
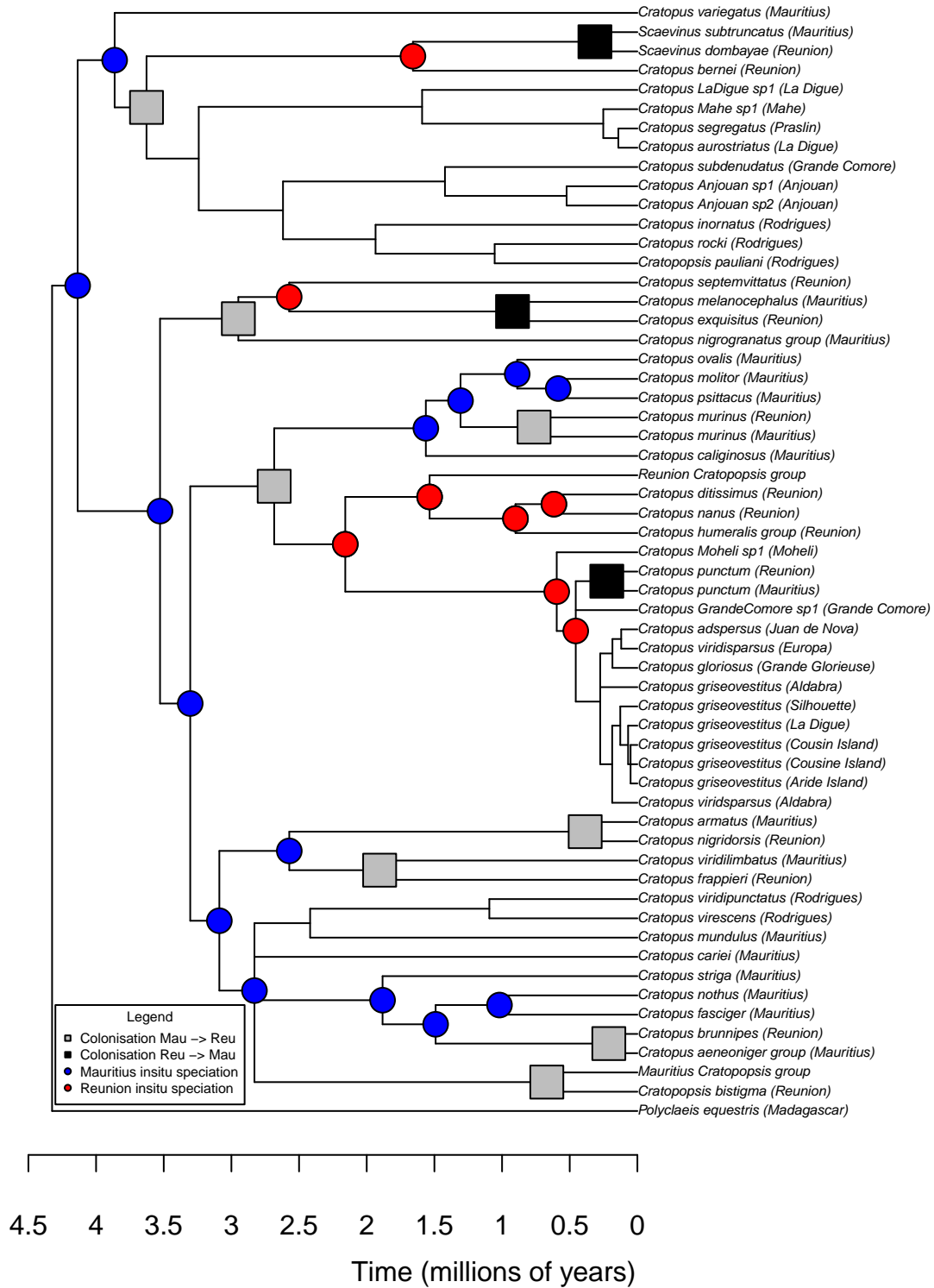
Statistical analysis of node ages

For each of the statistical analyses outlined in the main manuscript (except for where explicitly noted) we have taken a bootstrapping approach that allows us to compare the mean age of our nodes of interest (*i.e.* a comparison of colonisation node ages across islands, *in situ* speciation nodes vs colonisation node ages for each island and flight loss nodes vs all other nodes) to an equally sized subset of the remaining nodes in each analysis resampled 1000 times. In each iteration, any given node was only sampled once and in each histogram, the vertical red line indicates the mean or single value that the resampled node ages are being tested against.

S3.1 Node age statstics based on the ancestral state estimation using Bayesian Binary Markov analysis in RASP.

S3.1.1 RASP BBM ancestral state reconstruction.

From the previous plot we can group nodes as indicated in the legend.

Cratopus variegatus (Mauritius)
Scaevinus subtruncatus (Mauritius)
Scaevinus dombayae (Reunion)
Cratopus bernei (Reunion)
Cratopus LaDigue sp1 (La Digue)
Cratopus Mahe sp1 (Mahe)
Cratopus segregatus (Praslin)
Cratopus aurostriatus (La Digue)
Cratopus subdenudatus (Grande Comore)
Cratopus Anjouan sp1 (Anjouan)
Cratopus Anjouan sp2 (Anjouan)
Cratopus inornatus (Rodrigues)
Cratopus rocki (Rodrigues)
Cratopopsis pauliani (Rodrigues)
Cratopus septemvittatus (Reunion)
Cratopus melanocephalus (Mauritius)
Cratopus exquisitus (Reunion)
Cratopus nigrogranatus group (Mauritius)
Cratopus ovalis (Mauritius)
Cratopus molitor (Mauritius)
Cratopus psittacus (Mauritius)
Cratopus murinus (Reunion)
Cratopus murinus (Mauritius)
Cratopus caliginosus (Mauritius)
Reunion Cratopopsis group
Cratopus ditissimus (Reunion)
Cratopus nanus (Reunion)
Cratopus humeralis group (Reunion)
Cratopus Moheli sp1 (Moheli)
Cratopus punctum (Reunion)
Cratopus punctum (Mauritius)
Cratopus GrandeComore sp1 (Grande Comore)
Cratopus adspersus (Juan de Nova)
Cratopus viridisparsus (Europa)
Cratopus gloriosus (Grande Glorieuse)
Cratopus griseovestitus (Aldabra)
Cratopus griseovestitus (Silhouette)
Cratopus griseovestitus (La Digue)
Cratopus griseovestitus (Cousin Island)
Cratopus griseovestitus (Cousine Island)
Cratopus griseovestitus (Aride Island)
Cratopus viridisparsus (Aldabra)
Cratopus armatus (Mauritius)
Cratopus nigridorsis (Reunion)
Cratopus viridilimbatus (Mauritius)
Cratopus frappieri (Reunion)
Cratopus viridipunctatus (Rodrigues)
Cratopus virescens (Rodrigues)
Cratopus mundulus (Mauritius)
Cratopus cariei (Mauritius)
Cratopus striga (Mauritius)
Cratopus nothus (Mauritius)
Cratopus fasciger (Mauritius)
Cratopus brunnipes (Reunion)
Cratopus aeneoniger group (Mauritius)
Mauritius Cratopopsis group
Cratopopsis bistigma (Reunion)
Polyclaeis equestris (Madagascar)

Legend
■ Colonisation Mau –> Reu
■ Colonisation Reu –> Mau
● Mauritius insitu speciation
● Reunion insitu speciation

4.5   4   3.5   3   2.5   2   1.5   1   0.5   0

Time (millions of years)

S3.1.2 Comparing the average age of colonisation nodes on Réunion to those Mauritius

Histogram of mauritian vs Réunion colonisation ages
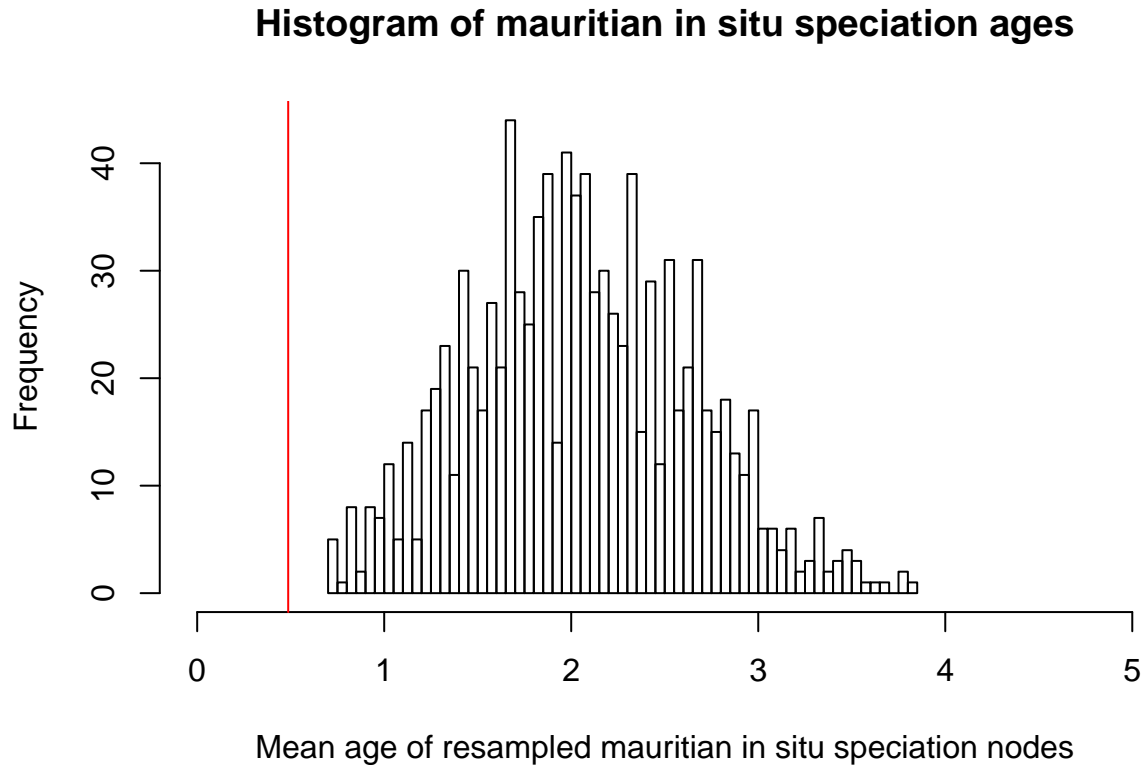

Mean age of resampled Réunion colonisation nodes

```
## 
##   One Sample t-test
## 
## data:  mean.Reu.col
## t = 61.219, df = 999, p-value < 2.2e-16
## alternative hypothesis: true mean is not equal to 0.4866667
## 95 percent confidence interval:
##  1.633682 1.709651
## sample estimates:
## mean of x
##  1.671667
```

The age of nodes leading to colonisations of Mauritius (*mean age* = 0.49 mya) is significantly less than nodes leading to colonisations of Réunion (*mean age* = 1.67 mya, *95 % CI* = 1.63 mya - 1.71 mya, *t* = 61.22, *df* = 999, *p* < 0.0001)

S3.1.3 Comparing the age of colonisation events on Mauritius to *in situ* speciation events

This is reversed relative to the test performed on Réunion nodes as there are many more *in situ* speciation nodes on Mauritius than colonisations.

Histogram of mauritian in situ speciation ages

Mean age of resampled mauritian in situ speciation nodes

```
##
##   One Sample t-test
##
## data:  mean.Mau.insitu
## t = 83.763, df = 999, p-value < 2.2e-16
## alternative hypothesis: true mean is not equal to 0.4866667
## 95 percent confidence interval:
##   2.016562 2.089964
## sample estimates:
## mean of x
##   2.053263
```

Here we can see that nodes leading to colonisations of Mauritius (*mean age* = 0.49 mya) are significantly younger than *in situ* speciation events (*mean age* = 2.05 mya, *95 % CI* = 2.02 mya - 2.09 mya, *t* = 83.76, *df* = 999, *p* < 0.0001)

S3.1.4 Comparing the age of *in situ* speciation events on Réunion to colonisation events

Histogram of Réunion colonisation ages

Mean age of resampled Réunion colonisation nodes
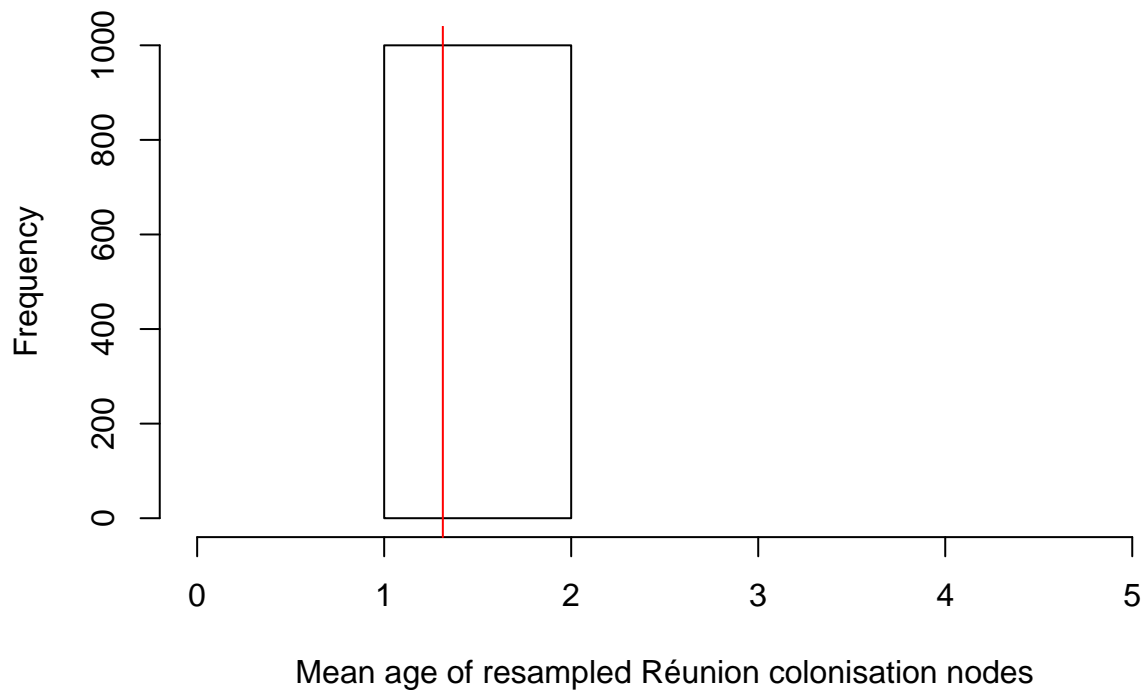
As there are exactly the same number of colonisation and *in situ* speciation events inferred for Réunion, resampling does not make sense as it will repeatedly return the same mean value. A more apropriate test in this situation is the age of all colonisation nodes vs all *in situ* speciation nodes.

```
##
##   Welch Two Sample t-test
##
## data:  Reu.col$Median_age and Reu.insitu$Median_age
## t = 0.61709, df = 11.448, p-value = 0.5493
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.8541646  1.5241646
## sample estimates:
## mean of x mean of y
##   1.64875   1.31375
```

Colonisations of Réunion (*mean age* = 1.31 mya, *sd* = 1.32) are not significantly different in age to *in situ* speciation nodes on Réunion (*mean age* = 1.65 mya, *sd* = 0.79, t = 0.62, df = 11.45, p = 0.55)

S3.1.5 Comparing the age of flight loss nodes to nodes without flight loss

Histogram of flightloss vs normal node ages

Mean age of resampled flight capable nodes
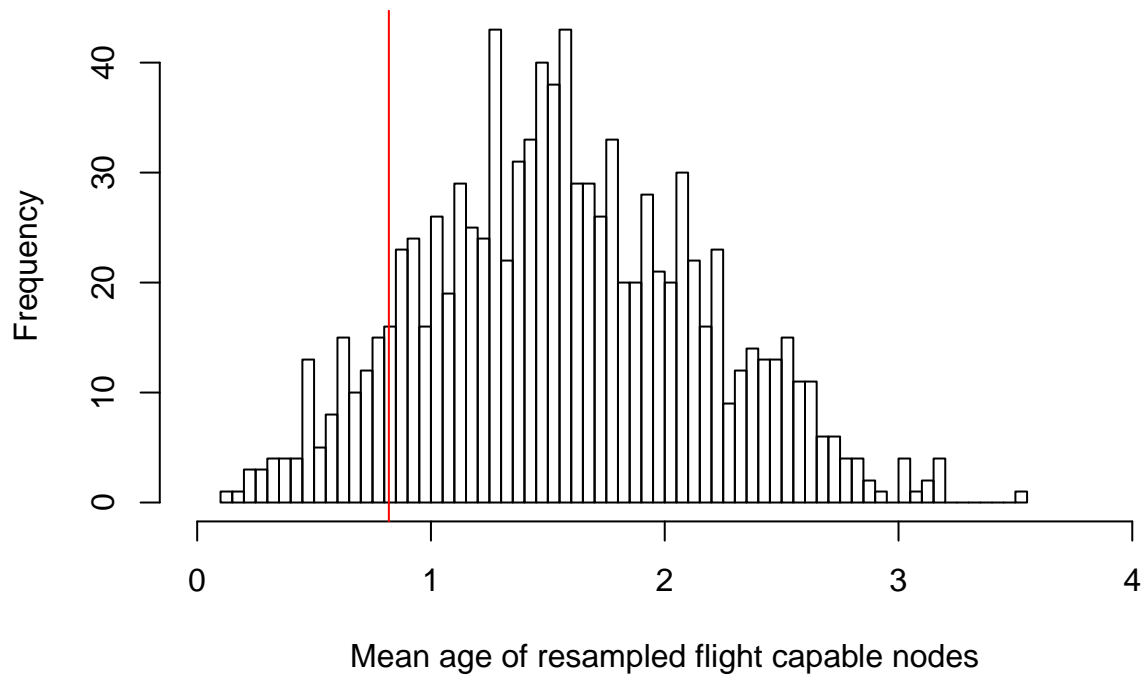
```
##
##  One Sample t-test
##
## data:  mean.fl
## t = 39.933, df = 999, p-value < 2.2e-16
## alternative hypothesis: true mean is not equal to 0.82
## 95 percent confidence interval:
##  1.535118 1.609032
## sample estimates:
## mean of x
##  1.572075
```

Nodes leading to flight loss (*mean age* = 0.82 mya) are significantly younger than other nodes (*mean age* = 1.57 mya, *95 % CI* = 1.54 mya - 1.61 mya, *t* = 39.93, *df* = 999, *p* < 0.0001)

S3.2 Node age statstics based on the ancestral state estimation using Maximum Likelihood analyses in R.

S3.2.1 R Maximum Likelihood ancestral state reconstruction.
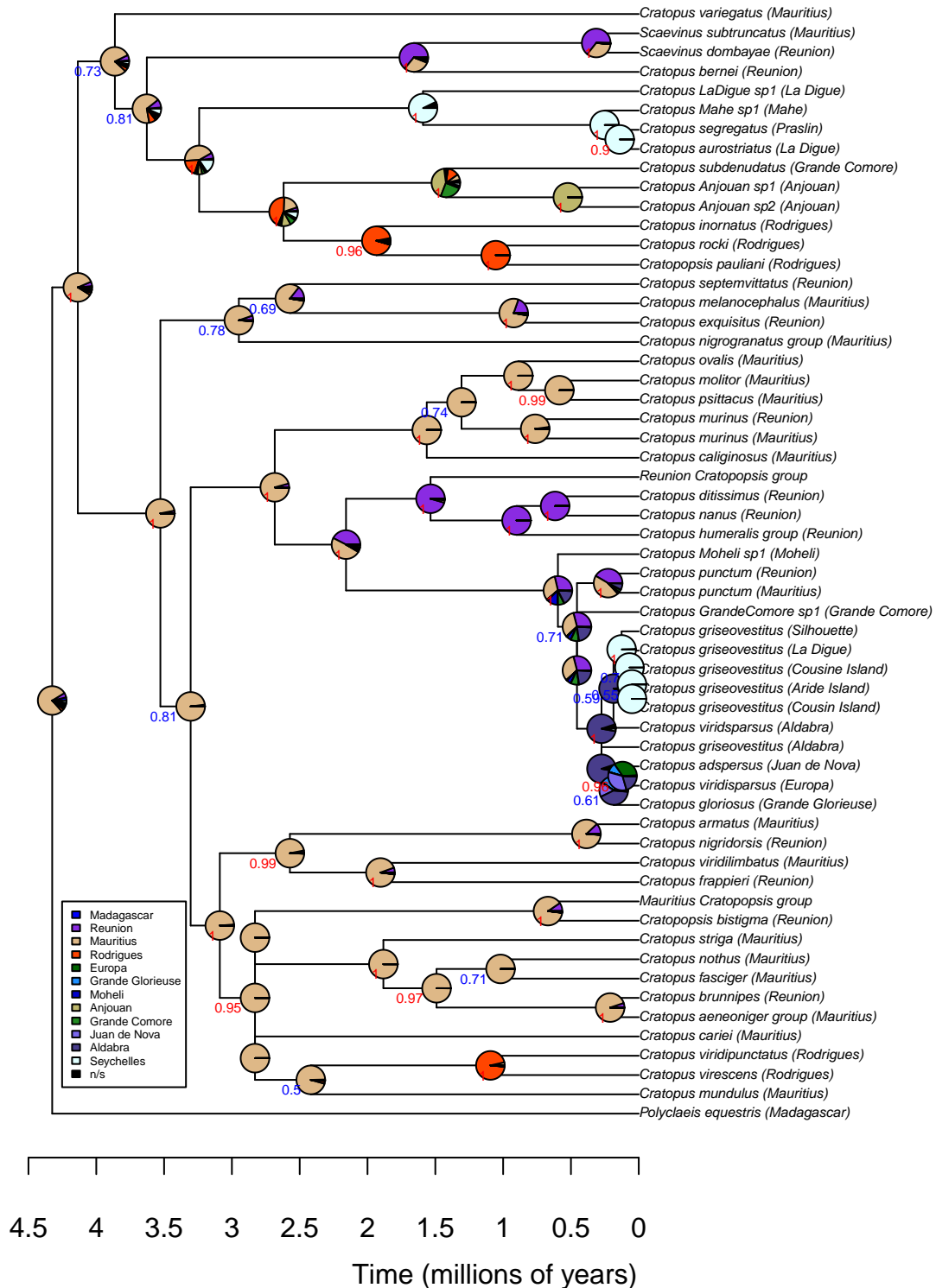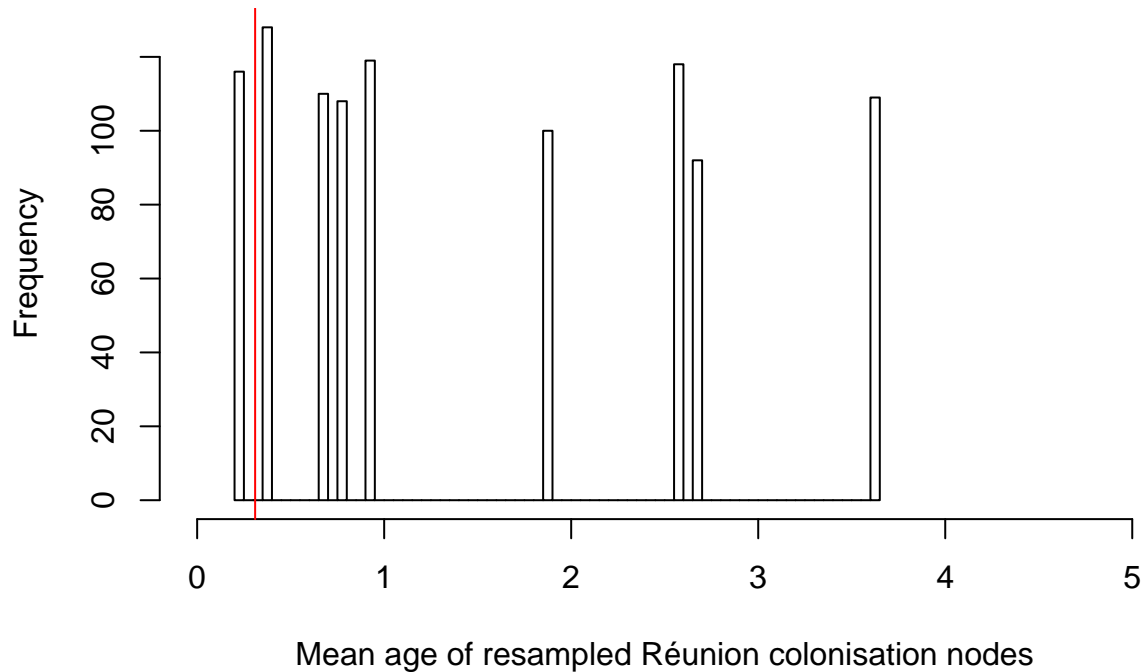
From the previous plot we can group nodes as indicated in the legend.
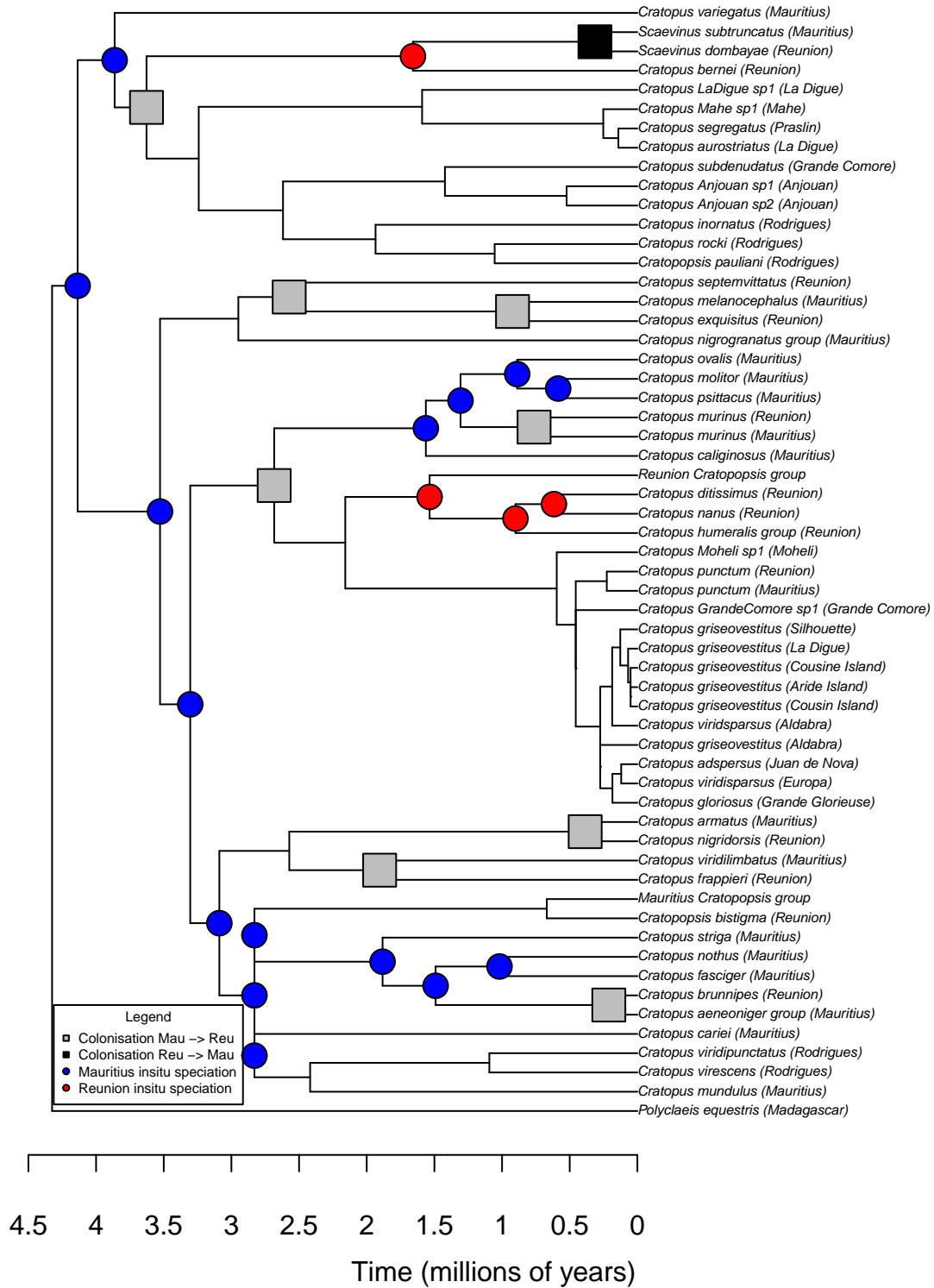
S3.2.2 Comparing the average age of colonisation nodes on Réunion to those Mauritius

Histogram of Réunion colonisation ages

Mean age of resampled Réunion colonisation nodes

As can be seen here, with only one colonisation of Mauritius from Réunion inferred, the script repeatedly samples single node ages, a more appropriate test in this instance is all Réunion colonisations vs the age of the single Mauritian colonisation.

```
##
##   One Sample t-test
##
## data:  Reu.col$Median_age
## t = 3.0158, df = 8, p-value = 0.01667
## alternative hypothesis: true mean is not equal to 0.31
## 95 percent confidence interval:
##   0.596078 2.455033
## sample estimates:
## mean of x
##   1.525556
```

The age of the single colonisation of Mauritius (0.31 mya) is significantly less than nodes leading to colonisations of Réunion (*mean age* = 1.53 mya, *95 % CI* = 0.6 mya - 2.46 mya, *t* = 3.02, *df* = 8, *p* = 0.02)

S3.2.3 Comparing the age of colonisation events on Mauritius to *in situ* speciation events

This is reversed relative to the test performed on Réunion nodes as there are many more *in situ* speciation nodes on Mauritius than colonisations.

Histogram of mauritian in situ speciation ages

Mean age of resampled mauritian in situ speciation nodes
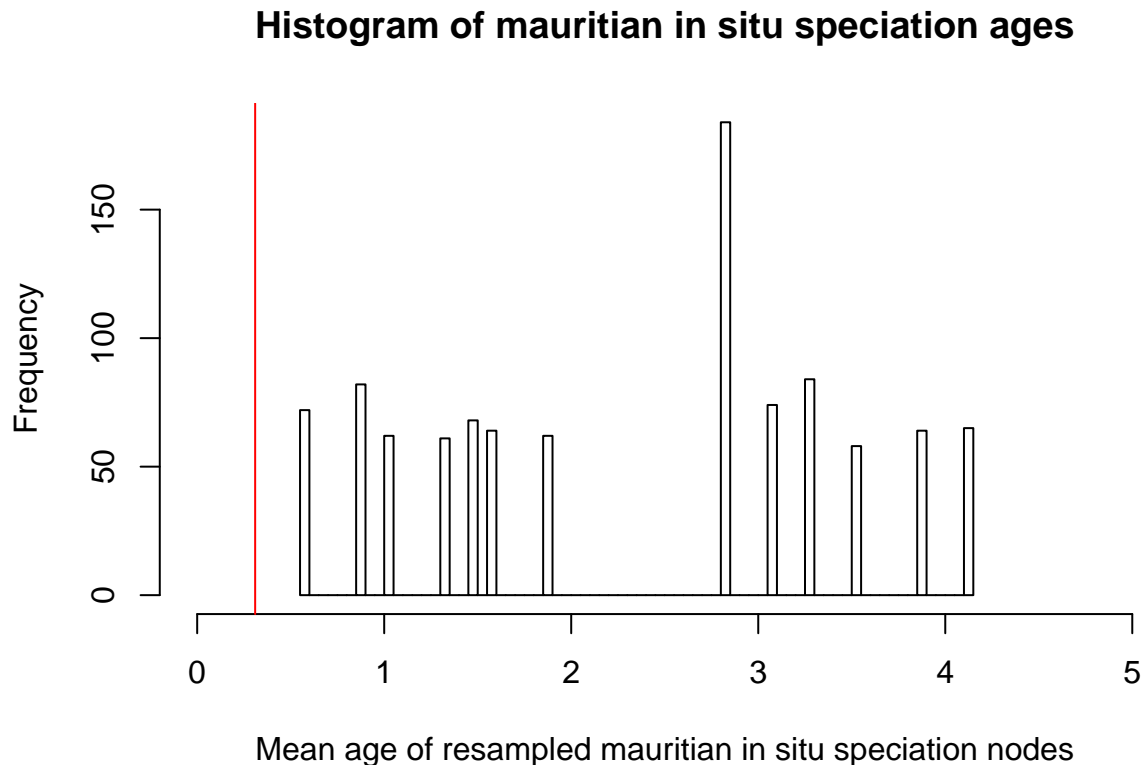
As with the t-test for Réunion vs Mauritian colonisation ages there is only one colonisation of Mauritius from Réunion inferred. So once again, a more appropriate test is the age of all Mauritian *in situ* speciation nodes vs the age of the single Mauritian colonisation.

```
##
##  One Sample t-test
##
## data:  Mau.insitu$Median_age
## t = 6.8173, df = 14, p-value = 8.361e-06
## alternative hypothesis: true mean is not equal to 0.31
## 95 percent confidence interval:
##  1.703627 2.983040
## sample estimates:
## mean of x
##  2.343333
```

Here we can see that the single colonisation of Mauritius (0.31 mya) is significantly younger than the *in situ* speciation events on Mauritius (*mean age* = 2.34 mya, *95 % CI* = 1.7 mya - 2.98 mya, *t* = 6.82, *df* = 14, *p* < 0.0001)

S3.2.4 Comparing the age of *in situ* speciation events on Réunion to colonisation events

Histogram of Réunion colonisation ages



Mean age of resampled Réunion colonisation nodes
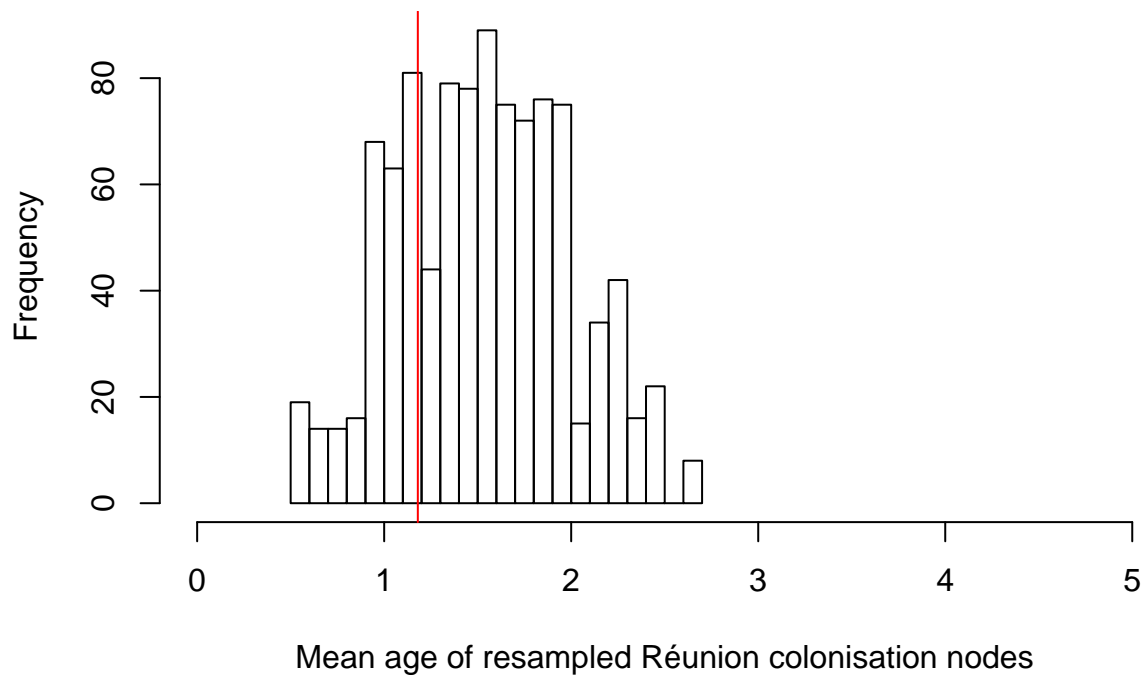
```
##
##   One Sample t-test
##
## data:  mean.Reu.col
## t = 8.1824, df = 999, p-value = 8.413e-16
## alternative hypothesis: true mean is not equal to 1.18
## 95 percent confidence interval:
##   1.404274 1.545786
## sample estimates:
## mean of x
##   1.47503
```

In situ speciation events on Réunion (*mean age* = 1.18 mya) are significantly younger than colonisation events on Réunion (*mean age* = 1.48 mya, *95 % CI* = 1.4 mya - 1.55 mya, *t* = 8.18, *df* = 999, *p* < 0.0001)

###S3.2.5 Comparing the age of flight loss nodes to nodes without flight loss

Histogram of flightloss vs normal node ages

Mean age of resampled flight capable nodes
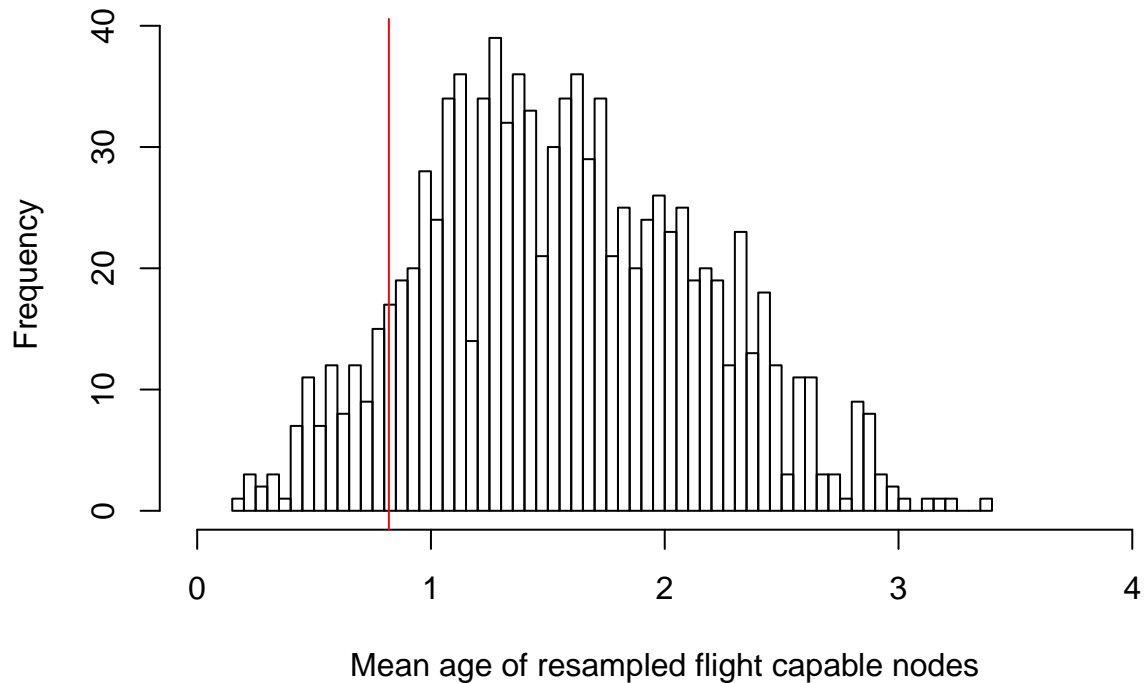
```
##
##  One Sample t-test
##
## data:  mean.fl
## t = 40.109, df = 999, p-value < 2.2e-16
## alternative hypothesis: true mean is not equal to 0.82
## 95 percent confidence interval:
##  1.529678 1.602692
## sample estimates:
## mean of x
##  1.566185
```

Nodes leading to flight loss (*mean age* = 0.82 mya) are significantly younger than other nodes (*mean age* = 1.57 mya, *95 % CI* = 1.53 mya - 1.6 mya, *t* = 40.11, *df* = 999, *p* < 0.0001)