

# DateLife Workflows

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## Taxon Anolis

### 1. Query source chronograms

There are 458 subspecies in the Open Tree of Life Taxonomy for the taxon Anolis. Information on time of divergence is available for 302 of these species across 6 published and peer-reviewed chronograms. Original study citations as well as number of Anolis species found across those source chronograms is shown in Table 1. All source chronograms are fully ultrametric and their maximum ages range from 47.843 to 72.578 million years ago (MYA). As a means for comparison, lineage through time plots of all source chronograms available in data base are shown in figure 1.

### 2. Summarize results from query

LTT plots are a nice way to visually compare several trees. But what if you want to summarize information from all source chronograms into a single summary chronogram?

The first step is to identify the degree of species overlap among your source chronograms: if each source chronogram has a unique sample of species, it will not be possible to combine them into a single summary chronogram. To identify the set of trees or *grove* with the most source chronograms that have at least two overlapping taxa, we followed Ané et al. 2016. In this case, not all source chronograms found for the Anolis have at least two overlapping species. The largest grove has 2 chronograms (out of 6 total source chronograms).

Now that we have identified a grove we can go on to summarize it by translating the source chronograms into patristic distance matrices and then averaging them into a single summary matrix; yes, this first step is *that* straightforward. We can average the source matrices by simply using the mean or median distances, or we can use methods that involve transforming the original distance matrices –such as the super distance matrix (SDM) approach of Criscuolo et al. 2006– by minimizing the distances across source matrices. As a result of such transformation, an SDM summary matrix can contain negative values. In this case, the SDM summary matrix has some negative values in the following taxa: *Anolis porcus*, *Anolis guamuhaya*, *Chamaeleolis chameleontides*, *Chamaeleolis chameleontides*.

Because our summary matrix is basically a distance matrix, a distance-based clustering algorithm could be used to reconstruct the tree. Algorithms such as neighbour joining (NJ) and unweighted pair group method with arithmetic mean (UPGMA) are fast and work very well when there are no missing values in the matrices. However, summary matrices coming from source chronograms usually have several NAs and missing rows. When this happens, variants of traditional clustering algorithms have been developed to deal with missing values. However, even these methods do not work well with our summary matrices, as shown in the following section. We should note that these clustering methods are usually applied to distance matrices representing substitution rates and not absolute time.

## 2.1. Clustering a summary matrix

NJ, UPGMA, BIONJ, minimum variance reduction (MVR) and the triangle method (TM) algorithms were used to cluster median and SDM summary distance matrices. None of these clustering algorithms returned trees matching source chronograms (Fig. 2, Appendix Fig. ??). UPGMA is the only algorithm that returns ultrametric trees, but they are considerably older than expected from ages observed in source chronograms. The other methods returned trees with ages that coincide with those observed in source chronograms. However, they resulting chronograms are not ultrametric. To overcome the issues presented by clustering algorithms, we used all data available in the summary matrix as calibrations over a consensus tree to obtain a summary chornogram.

## 2.2. Calibrating a consensus tree with data from a summary matrix

Even if the branch lengths coming form the clustered chronograms are not adequate, the topology can still be used as a backbone tree that can be dated using data from the summary matrix as secondry calibrations. A summary of divergence times available for each node can be obtained from the summary matrix, simply by getting the nodes from the backbone tree that correspond to each pair of taxa in the matrix. Finally, this summary of node divergence times can be used with the consensus tree as input in any dating software that does not require data. The branch length aduster (BLADJ) algorithm [Webb2000] is really fast and does not make any evolutionary assumptions on age distribution. Other software such as MrBayes and r8s can be used instead of BLADJ by running them without data. In here, we show summary chronograms obtained using minimum, mean and maximum distances from the summary of node divergence times of the backbone tree as fixed ages in BLADJ (Fig. 3). Summary chronograms from both types of summary matrices are quite similar. As expected, SDM chronograms using minimum, mean and maximum distances do not vary much in their maximum age, because ages are transformed to minimize the variance. In contrast, the median chronograms obtained with minimum, mean and maximum distances have wider variation in their maximum ages, as can be observed in the distance between the green arrows in Fig. 3. This variation simply represents variation in source data.

## 3. Generate new chronograms

Another way to leverage information from the source chronograms is to use their node ages as secondary calibration points to date any tree topology (with or without branch lengths) given that at least two taxa from source chronograms are in the tips of that topology. In this data set we have 1183 calibrations in total (that basically corresponds to the sum of the number of nodes from each source chronogram). Once we have a target tree topology, we can map the calibrations to the target tree. Some nodes will have several calibrations and some others might have none. Also, some node ages can be conflicting, with descendant nodes being older than parent nodes. We performed a series of cross validation analyses with different dating methods, by dating the topologies of each source chronogram using information from all other source chronograms as calibration points.

### 3.1. Calibrate a tree without branch length data

To date a tree in the absence of data on relative evolutionary rates (molecular or morphological) we follow the same methodology as the one used to obtain summary chronograms. First, we obtained the nodes that correspond to each pair of taxa in the data set of total calibrations to construct a summary of node calibrations for the backbone tree. Then, we used mean ages as secondary calibrations for the backbone tree with the software BLADJ. In general, the time of divergence information from other source chronograms allows to recover the divergence times from the original study. In some cases, it is evident that information from a particular study really affects the summary of divergence times. In some other cases, the root of the tree is not calibrated. Since BLADJ has no underlying model of evolution, there is no way for the algorithm to calculate this age. To fix this, we simply added a unit of the mean difference across ranked ages from secondary calibrations (Fig. 4).

### **3.2. Calibrate a tree with data**

If you have a tree with branch lengths proportional to relative substitution rates, you can use the source chronogram node ages as secondary calibrations with various algorithms for phylogenetic dating to get branch lengths proportional to absolute time. To exemplify this, we got DNA markers from the Barcode of Life Database (BOLD) to estimate branch lengths as relative DNA substitution rates on a tree topology of our choosing. In this example we retrieved data from the cytochrome C oxidase subunit I (COI) marker, that is of widespread use in barcoding, providing DNA data for a very wide number of organisms. A tree with branch lengths could be constructed for 6 source chronograms (out of 6) available for the Anolis. To date these trees we are using the software PATHd8 for tree dating without a molecular clock model, using calibrations from all other source chronograms. Sometimes, calibrations conflict between them. To deal with conflicting calibrations, we can either expand them to make them agree, or we can congruify them to the topology of the tree to be dated. Results from both approaches are shown in the following two sections.

#### **3.2.1. Expanding calibrations**

#### **3.2.2. Summarizing calibrations (congruifying calibrations)**

## 4. Example with subspecies tree

As an example, we're gonna date the subspecies tree of the group using all approaches for generating new data.

Now, let's say you like the Open Tree of Life Taxonomy and you want to stick to that tree. Dates from available studies were tested over the Open Tree of Life Synthetic tree of Anolis and a tree was constructed, but all branch lengths are NA. We also tried each source chronogram independently, with the Dated OToL and with each other, as a form of cross validation in Table 2. This is not working perfectly yet, but we are developping new ways to use all calibrations efficiently.

# Tables and Figures

Table 1: Anolis source chronogram studies information.

	<i>Citation</i>	<i>Source N</i>	<i>Taxon N</i>
1.	Hedges, S. Blair, Julie Marin, Michael Suleski, Madeline Paymer, Sudhir Kumar. 2015. Tree of life reveals clock-like speciation and diversification. <i>Molecular Biology and Evolution</i> 32 (4): 835-845	1	192/458
2.	Mahler, D. L., T. Ingram, L. J. Revell, J. B. Losos. 2013. Exceptional Convergence on the Macroevolutionary Landscape in Island Lizard Radiations. <i>Science</i> 341 (6143): 292-295.	1	98/458
3.	Pyron, R. Alexander, Frank T. Burbrink. 2013. Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. <i>Ecology Letters</i> 17 (1): 13-21	1	205/458
4.	Steven Poe, Adrián Nieto-montes de oca, Omar Torres-carvajal, Kevin De Queiroz, Julián A. Velasco, Brad Truett, Levi N. Gray, Mason J. Ryan, Gunther Köhler, Fernando Ayala-varela, Ian Latella, 2017, 'A Phylogenetic, Biogeographic, and Taxonomic study of all Extant Species of Anolis (Squamata; Iguanidae)', <i>Systematic Biology</i> , vol. 66, no. 5, pp. 663-697	1	289/458
5.	Wright, April M., Kathleen M. Lyons, Matthew C. Brandley, David M. Hillis. 2015. Which came first: The lizard or the egg? Robustness in phylogenetic reconstruction of ancestral states. <i>Journal of Experimental Zoology Part B: Molecular and Developmental Evolution</i> 324 (6): 504-516	1	203/458
6.	Yuchi Zheng, John J. Wiens, 2016, 'Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species', <i>Molecular Phylogenetics and Evolution</i> , vol. 94, pp. 537-547	1	202/458

**Source N:** Number of source chronograms reported in study.

**Taxon N:** Number of queried taxa found in source chronograms.

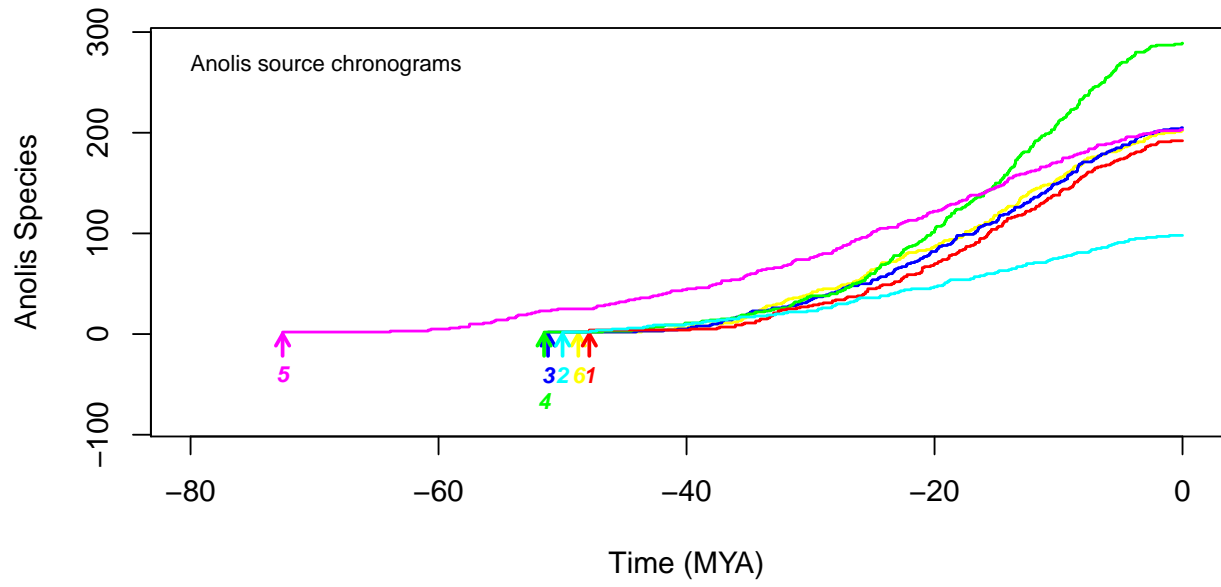


Figure 1: Lineage through time (LTT) plots of source chronograms available in data base for species in the Anolis. Numbers correspond to original studies in Table 1. Arrows indicate maximum age of each chronogram.

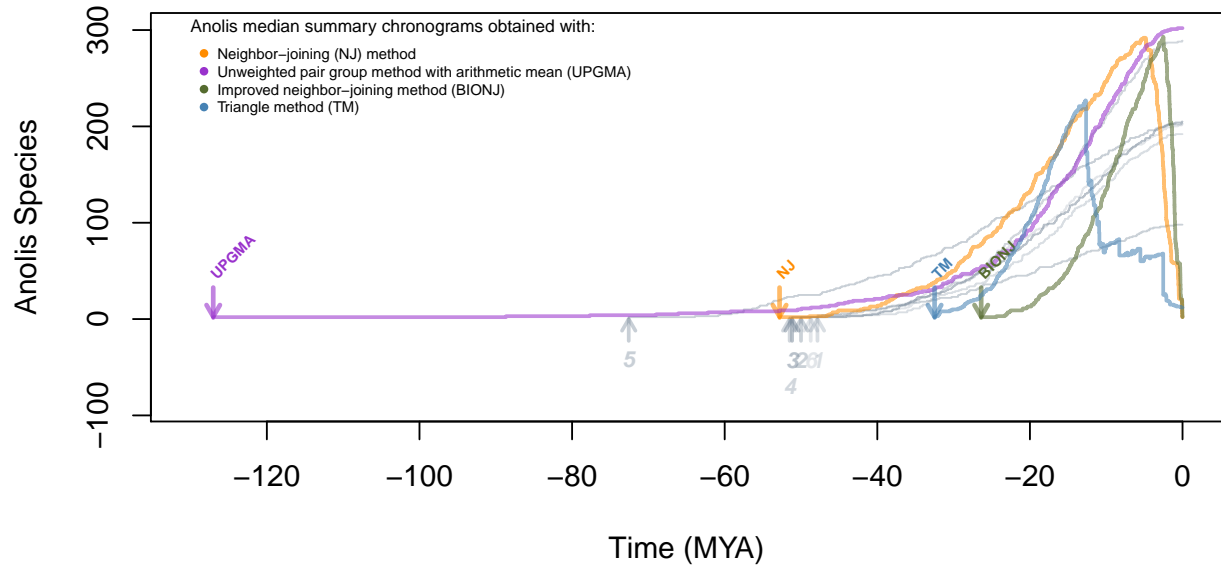


Figure 2: Lineage Through Time plots of Anolis median summary chronograms obtained with different clustering algorithms. Not all algorithms worked with this summary matrix and we are only showing here the ones that worked. Chronograms obtained from the SDM summary matrix are very similar to the ones from the median summary matrix with all clustering algorithms (Appendix Fig. ??).

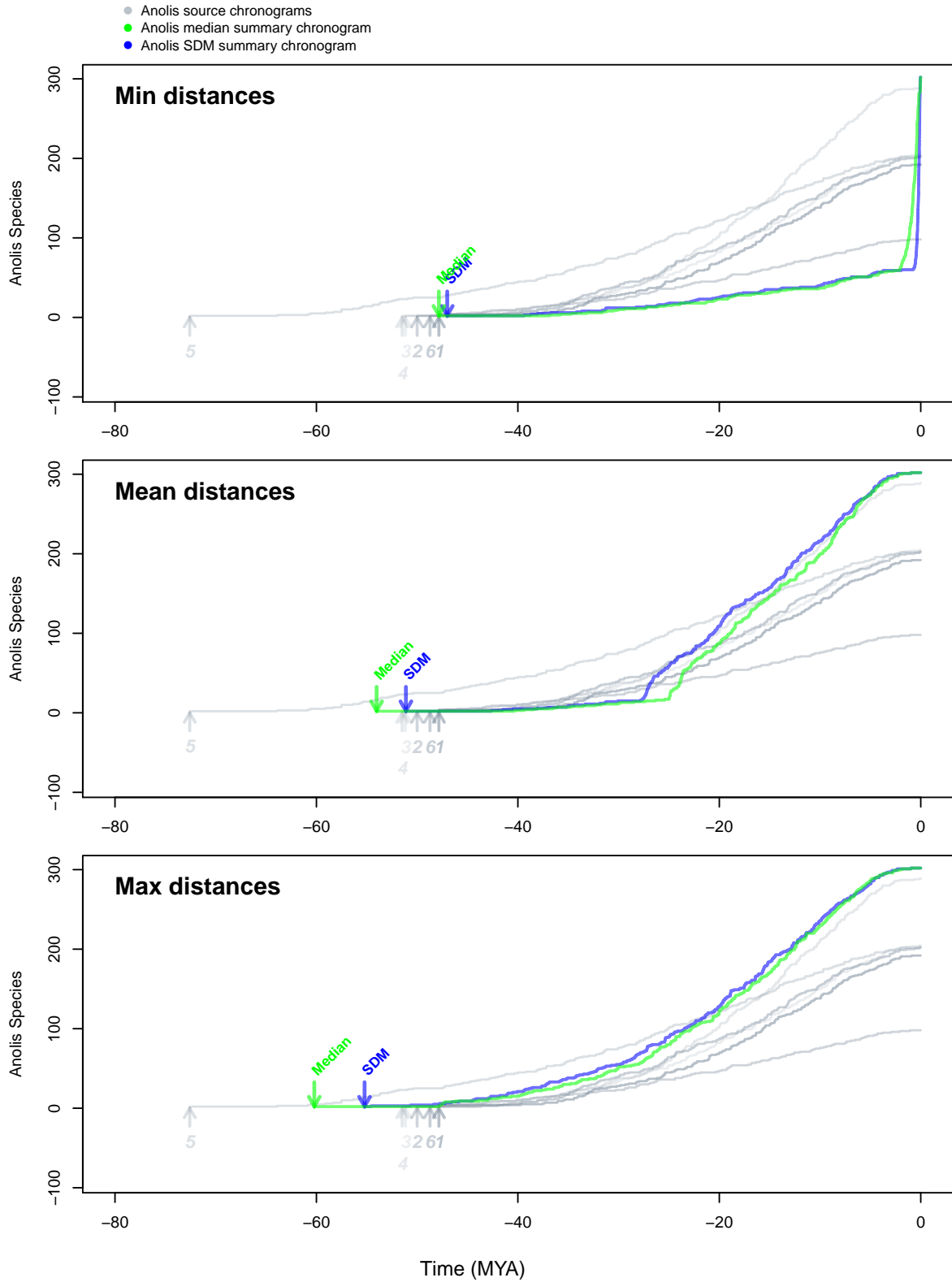


Figure 3: Anolis lineage through time (LTT) plots from source chronograms (gray), median (green) and SDM (blue) summary chronograms obtained by calibrating a consensus tree topology with distance data from respective summary matrices and then adjusting branch lengths with BLADJ.



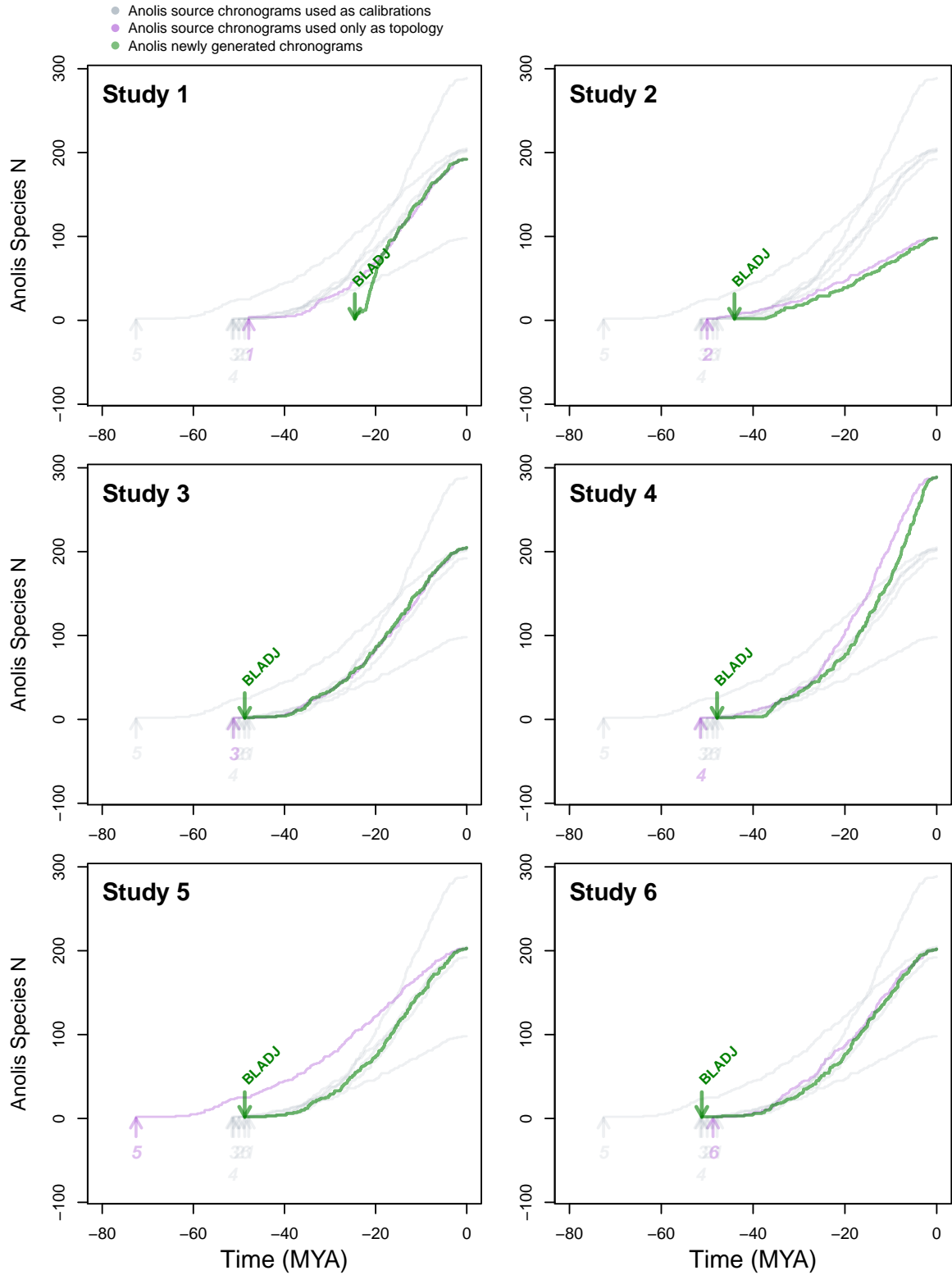


Figure 4: Anolis lineage through time (LTT) plots from source chronograms used as secondary calibrations (gray), source chronograms used as topology (purple) and chronograms resulting from calibrating the latter with the former, using BLADJ (green).

## Appendix

The following species were not found in the chronogram database: *Anolis alocomyos*, *Anolis altavelensis*, *Anolis anfiloquioi*, *Anolis anisolepis*, *Anolis attenuatus*, *Anolis bellipeniculus*, *Anolis birama*, *Anolis breedlovei*, *Anolis caquetae*, *Anolis carlliebi*, *Anolis carlostoddi*, *Anolis cf. alocomyos GK-2015*, *Anolis cf. humilis JJK-2013*, *Anolis cf. polylepis*, *Anolis charlesmyersi*, *Anolis chlorocyaneus*, *Anolis chrysops*, *Anolis concolor*, *Anolis cuscoensis*, *Anolis damulus*, *Anolis delafuentei*, *Anolis deltae*, *Anolis desiradei*, *Anolis dissimilis*, *Anolis eewi*, *Anolis fairchildi*, *Anolis forbesorum*, *Anolis fugitivus*, *Anolis gibbiceps*, *Anolis haguei*, *Anolis ibague*, *Anolis immaculogularis*, *Anolis impetigosus*, *Anolis incredulus*, *Anolis juangundlachi*, *Anolis kreutzi*, *Anolis laevis*, *Anolis lamari*, *Anolis landestoyi*, *Anolis leditzigorum*, *Anolis lemniscatus*, *Anolis limon*, *Anolis menta*, *Anolis microlepis*, *Anolis mirus*, *Anolis morazani*, *Anolis muralla*, *Anolis nasofrontalis*, *Anolis nietoi*, *Anolis nigrolineatus*, *Anolis osa*, *Anolis paravertebralis*, *Anolis philopunctatus*, *Anolis phyllorhinus*, *Anolis pigmaequestrus*, *Anolis pijolense*, *Anolis pinchoti*, *Anolis propinquus*, *Anolis pseudotigrinus*, *Anolis purpurescens*, *Anolis radulinus*, *Anolis rhombifer*, *Anolis rimarum*, *Anolis rivalis*, *Anolis roosevelti*, *Anolis ruibali*, *Anolis ruizi*, *Anolis rupinae*, *Anolis sacamecatensis*, *Anolis santamartae*, *Anolis scapularis*, *Anolis schiedei*, *Anolis schmidtii*, *Anolis sierramaestrae*, *Anolis simmonsii*, *Anolis solitarius*, *Anolis spectrum*, *Anolis squamulatus*, *Anolis stevepoei*, *Anolis tenorioensis*, *Anolis terueli*, *Anolis tetarii*, *Anolis toledo*, *Anolis umbrivagus*, *Anolis utowanae*, *Anolis vaupesianus*, *Anolis vascus*, *Anolis vicarius*, *Anolis villai*, *Anolis wampuensis*, *Anolis wattsi*, *Anolis wermuthi*, *Anolis williamsii*, *Anolis zapotecorum*, *Ctenonotus cybotes*, *Ctenonotus ferreus*, *Ctenonotus pulchellus*, *Norops alvarezdeltoroi*, *Norops anisolepis*, *Norops antonii*, *Norops baccatus*, *Norops birama*, *Norops bocourthii*, *Norops bouvierii*, *Norops breedlovei*, *Norops chrysolepis*, *Norops cobanensis*, *Norops concolor*, *Norops conspersus*, *Norops cumingii*, *Norops cuprinus*, *Norops cymbops*, *Norops damulus*, *Norops delafuentei*, *Norops dollfusianus*, *Norops exsul*, *Norops forbesi*, *Norops fungosus*, *Norops gibbiceps*, *Norops granuliceps*, *Norops haguei*, *Norops hobartsmithi*, *Norops ibague*, *Norops imias*, *Norops johnmeyeri*, *Norops lemniscatus*, *Norops lineatus*, *Norops lynchi*, *Norops macrolepis*, *Norops macrophallus*, *Norops maculiventris*, *Norops mariarum*, *Norops matudai*, *Norops microlepis*, *Norops milleri*, *Norops notopholis*, *Norops opalinus*, *Norops parvicirculatus*, *Norops pentaprion*, *Norops petersii*, *Norops pinchoti*, *Norops pygmaeus*, *Norops rhombifer*, *Norops rivalis*, *Norops salvini*, *Norops scapularis*, *Norops schiedei*, *Norops schmidtii*, *Norops simmonsii*, *Norops sulcifrons*, *Norops tolimensis*, *Norops utowanae*, *Norops vicarius*, *Norops villai*, *Norops vittigerus*, *Norops vociferans*

Dated induced subtree could not be obtained for the Anolis.

This taxon's SDM matrix has some negative values in the following taxa: *Anolis porcus*, *Anolis guamuhaya*, *Chamaeleolis chameleontides*, *Chamaeleolis chameleontides*. This taxon's Median matrix has NO negative values.

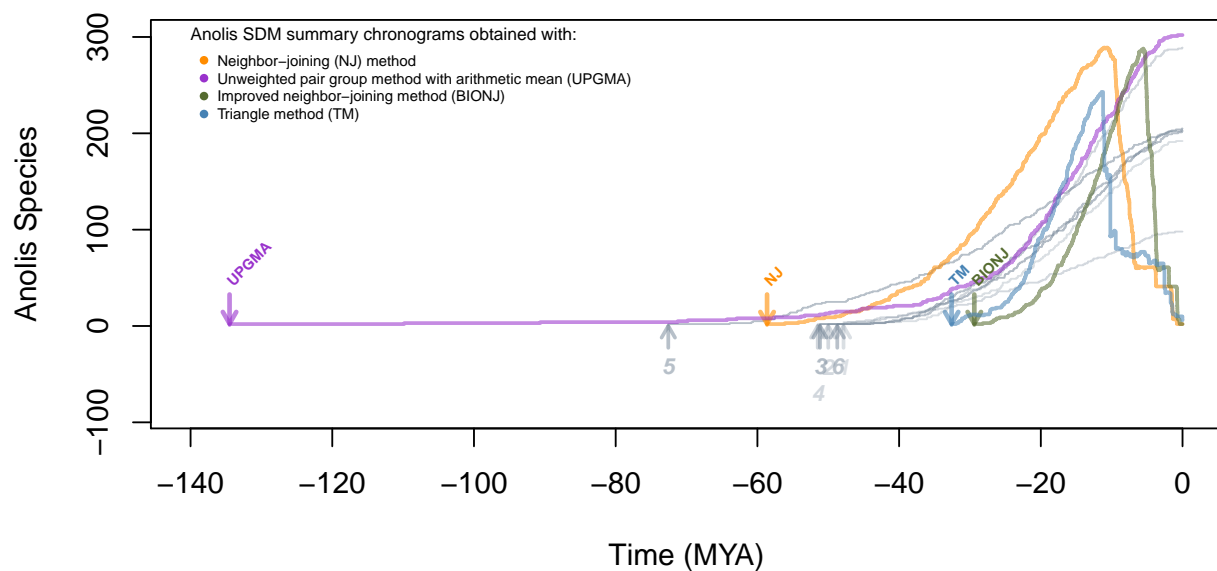


Figure 5: Lineage Through Time plots of Anolis SDM summary chronograms obtained with different clustering algorithms. Not all algorithms worked with the SDM summary matrix and we are only showing here the ones that worked. Chronograms obtained from the median summary matrix are very similar to the ones shown here with all algorithms (main figure 2).

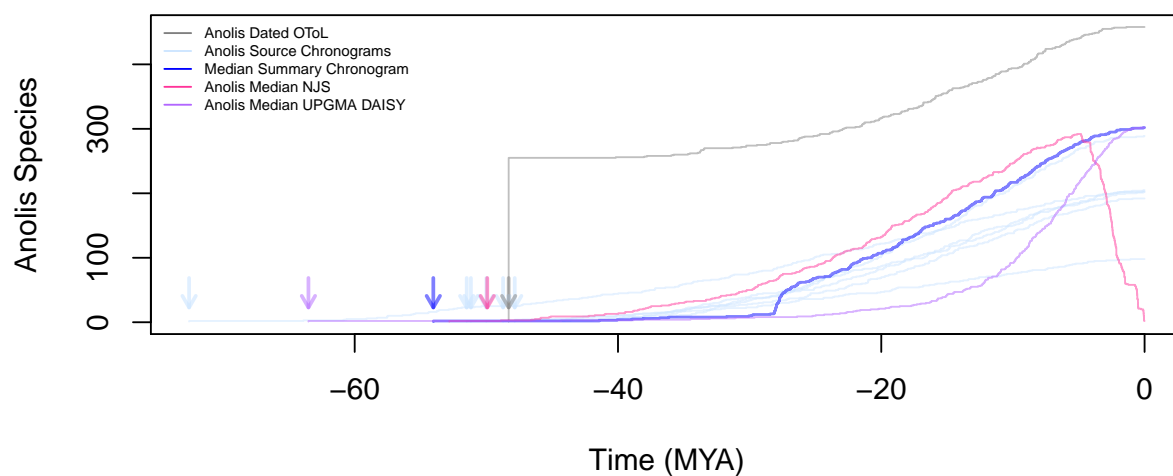


Figure 6: Anolis lineage through time (LTT) plots from source chronograms and Median summary matrix converted to phylo with different methods (NJ and UPGMA). Clustering algorithms used often are returning non-ultrametric trees or with maximum ages that are just off (too old or too young). So we developed an alternative algorithm in `datelife` to go from a summary matrix to a fully ultrametric tree.

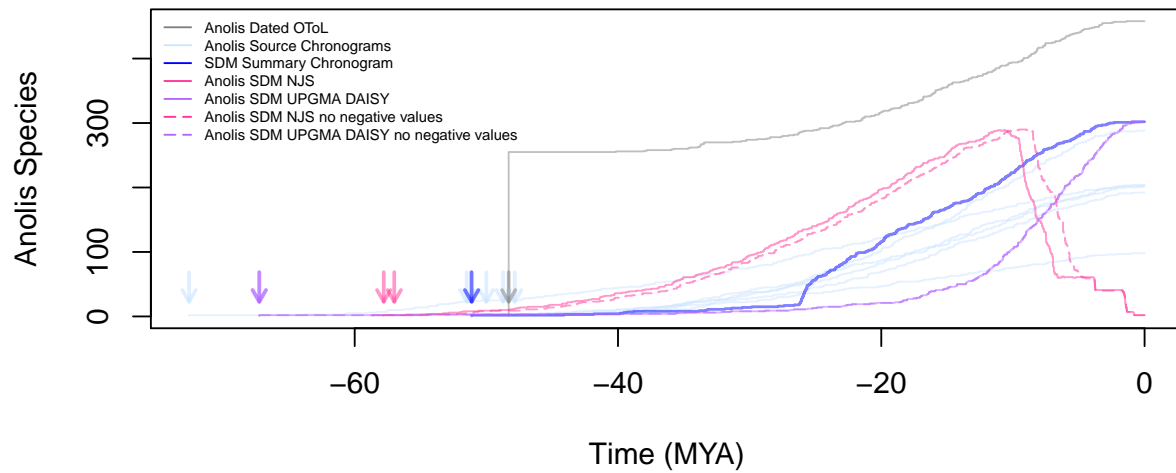


Figure 7: Anolis lineage through time (LTT) plots from source chronograms and SDM summary matrix converted to phylo with different methods (NJ and UPGMA). As you can note, dashed lines and solid lines from trees coming out from both types of clustering algorithms implemented are mostly overlapping. This means that removing negative values does not change results from clustering algorithms much. Clustering algorithms used often are returning non-ultrametric trees or with maximum ages that are just off (too old or too young). So we developed an alternative algorithm in **datelife** to go from a summary matrix to a fully ultrametric tree.

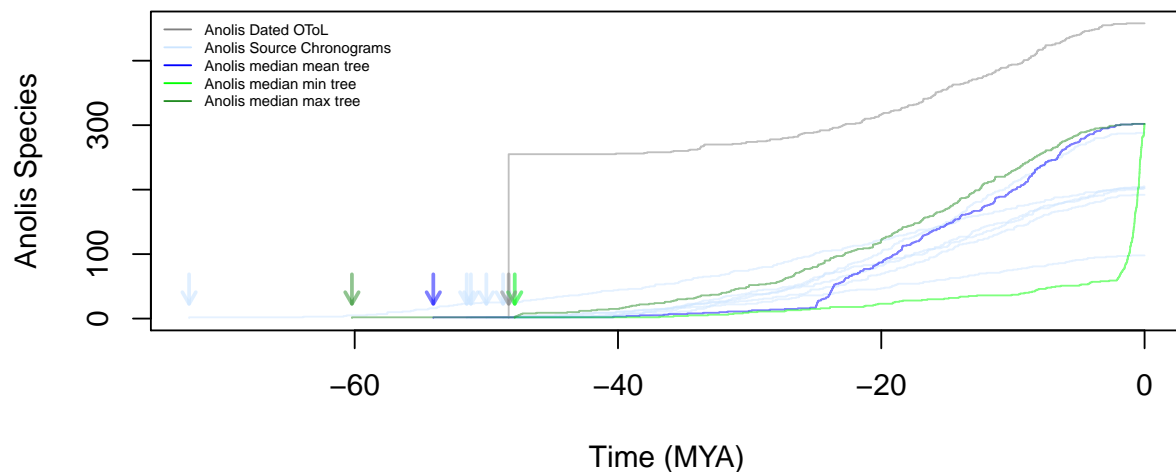


Figure 8: Anolis lineage through time (LTT) plots from source chronograms and Median summary matrix converted to phylo with **datelife** algorithm.

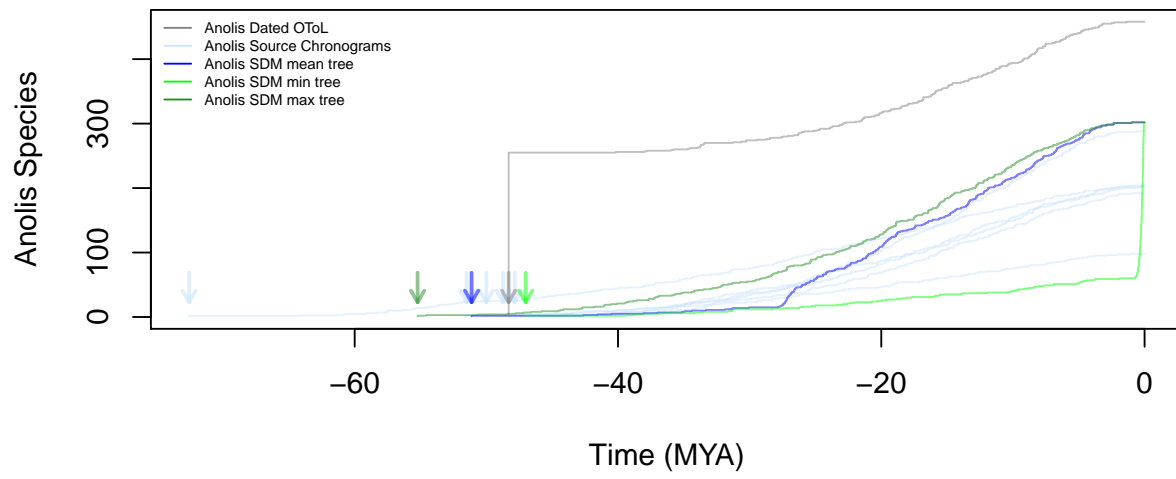


Figure 9: Anolis lineage through time (LTT) plots from source chronograms and SDM summary matrix converted to phylo with `datelife` algorithm.