**DiscoVista: interpretable visualizations of gene tree discordance**

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**Abstract**

Phylogenomics has ushered in an age of discordance. Analyses often reveal abundant discordances among phylogenies of different parts of genomes, as well as incongruences between species trees obtained using different methods or data partitions. Researchers are often left trying to make sense of such incongruences. Interpretive ways of measuring and visualizing discordance are needed, both among alternative species trees and gene trees, especially for specific focal branches of a tree. Here, we introduce DiscoVista, a publicly available tool that creates a suite of simple but interpretable visualizations. DiscoVista helps quantify the amount of discordance and some of its potential causes.

**Key words**— Software, Species tree estimation, Gene trees, Phylogenomics, Visualization, ILS

**1 Introduction**

The age of phylogenomics, once hoped to be the end of incongruence in phylogenetic analyses \( [\text{Rokas et al.}, 2003]\), has turned out to be ripe with incongruence \( [\text{Jeffroy et al.}, 2006]\) and methodological difficulties. In recent years, once mostly theoretical concerns about gene tree incongruence, incomplete lineage sorting (ILS), \( [\text{Maddison}, 1997]\) have proved of immense practical implication \( (\text{e.g., Jarvis et al.}, 2014; \text{Wickett et al.}, 2014)\). While methods that seek to address gene tree incongruence have been developed, no consensus has emerged as to the choice of the best methodology \( (\text{see debates in Edwards et al.}, 2016; \text{Springer and Gatesy}, 2016)\) for some examples). Nevertheless, a modern phylogenomics project has to at least consider the possibility of gene tree incongruence and its impacts, a feat made difficult by the noisy estimates of gene trees \( [\text{Springer and Gatesy}, 2016; \text{Patel et al.}, 2013; \text{Mirarab et al.}, 2016; \text{Sayyari and Mirarab}, 2016]\). Moreover, in some cases, the incongruence itself may be of interest \( [\text{Hahn and Nakhleh}, 2016]\).

Even ignoring gene tree discordance, choices of models to apply to the sequences, delineation of data partitions, alignment techniques, or simply software packages used to analyze the data have all proved consequential \( (\text{e.g., Jeffroy et al.}, 2006; \text{Jarvis et al.}, 2014; \text{Wickett et al.}, 2014; \text{Romiguier et al.}, 2013; \text{Philippel et al.}, 2011; \text{Zwickl et al.}, 2014)\).

These difficulties have compelled some researchers to use several alternative models and methods and then test the sensitivity of results to such choices. Occasionally, analysts choose to also perturb the set of species included, and they often run analyses on different partitions of the data. The analyst hopes for congruence between various analyses that would indicate robustness of the results to assumptions, but often observes differences. Ideally, the results of all analyses should be published, to convey the existence of incongruence in results to the reader.

As long as incongruence remains an important force in phylogenetics, we need interpretable ways to measure and visualize the discordance between species tree estimates resulting from different analytical method and assumptions, and also between gene trees and a summary species tree. Sophisticated tools have been developed to visualize discordance. For example, DensiTree \( [\text{Bouckaert}, 2010]\) overlays trees on top of each other to create a phylogenetic cloud, and SplitsTree \( [\text{Huson}, 1998]\) conveys incongruence by producing a network while keeping some of the tree-like structure. These tools create creative and striking indicators of discordance. Yet it is often hard to interpret the meaning of such figures in measurable ways. We believe that in addition to these methods, phylogenomics will benefit from
simple, interpretable, and easy-to-perform visualizations that help systematists to identify discordance and its potential causes.

2 Methods

In this paper, we introduce DiscoVista, a tool that creates a series of simple yet powerful visualizations of discordance. DiscoVista is a command-line tool and relies on several other packages, including Dendropy (Sukumaran and Holder 2010), ape (Paradis et al. 2004), newick utilities (Junier and Zdobnov 2010), the ggplot package (Wickham 2016), and ASTRAL (Sayyari and Mirarab 2016). The code, a Docker (Boettiger and Carl 2015) virtual image (for easy installation), and examples are available online at https://github.com/esayyari/DiscoVista.

DiscoVista strives for interpretability. In many analyses, not all branches in a phylogenetic tree are equally important because questions of interest typically concern several hypotheses surrounding the relationships between focal groups. Visualizing discordance with respect to only these focal relationships simplifies interpretation. Assessing hypotheses concerning these larger subsets of the species helps in answering the downstream biological questions of interest. Thus, DiscoVista allows researchers to define focal groups of taxa and evaluate discordance relevant to those groups. A central input to DiscoVista is a split definitions file (e.g., Figs. 2, 3a, and 3b), where the user can easily combine taxa into groups of interest and give the groups names (see supplementary material for details). Each split is a bipartition of the taxa into two groups and corresponds to an edge in an unrooted tree. The user can specify one side of a split (which would be a clade if the side that doesn’t include the root is given). With careful definition of splits, alternative hypotheses of interests could be specified.

DiscoVista generates several visualizations, and we describe the main ones below. Examples outputs are shown for a previously published phylotranscriptomic dataset of 107 plants (Wickett et al. 2014) (Figs. 1 and S3-S7). The exact commands for generating example figures are given in the supplementary materials.

i) Species tree compatibility (e.g., Figs. 2, S3, and S4): This visualization shows whether focal splits are supported, weakly rejected, or strongly rejected by different analyses. The inputs are a set of species trees in newick format, a support threshold, and the splits definition file; the output is a heat map. For each focal split and for each species tree, if the split is compatible with the tree, the corresponding cell is in shades of blue to green, and the spectrum of blue-green color indicates the branch support (any measure of support, including the bootstrap support, Bayesian posterior probability, or localPP (Sayyari and Mirarab 2016) values could be used.) A split that is incompatible with the fully resolved species tree, but is compatible with the tree when branches with low support values (specified by the passed threshold; e.g., below 75% BS) get contracted is considered weakly rejected.

Compatibility is a concept that we find useful for measuring discordance in an interpretable way. A split is compatible with an unrooted tree if it is compatible with all splits of that tree and can therefore be added to that tree; compatibility can be easily and efficiently checked (Warnow 1994). Let two splits be $A|B$ and $C|D$; then, the two splits are compatible if and only if one of the four pairwise intersections $A \cap C$, $A \cap D$, $B \cap C$, or $B \cap D$ is empty.

ii) Gene tree compatibility (e.g., Figs. 1, 2, S5, and S6): This visualization simply depicts the portion of gene trees supporting or rejecting each focal split. The inputs are one or several collections of gene trees, the split definition file, and a support threshold. For splits that are recovered in gene trees, branches with bootstrap support values above (below) a threshold are considered as highly (weakly) supported. Splits that are not in the original tree but are compatible with the tree if branches with low support values get contracted are considered as weakly rejected splits, and those that are not compatible even after contracting low support branches are considered strongly rejected. By default, gene trees that miss one side of the split completely are removed from the analyses. The figure can also be created such that genes that completely miss one side of the split (or completely miss a user-defined subset of a side of the split) are marked as “missing” (Fig. S5 and Fig. 1b).

iii) Branch quartet frequencies (Figs. 2 and 1a): Every internal branch of a species tree divides the tree into four parts, and thus, at least one quartet tree (often many) can be mapped uniquely onto that branch. For a given branch, assuming (a) the branch is correct (b) the only source of discordance between gene trees and species tree is ILS, and (c) there is no gene tree estimation error, the multi-species coalescent (MSC) model has specific expectations about these quartets (Allman et al. 2011). The probability of a gene tree quartet tree matching the species tree topology ($p > \frac{1}{4}$) is higher than probability of matching the two alternatives, and the two alternatives have equal probabilities ($q = \frac{1-p}{2} < \frac{1}{4}$).
Figure 1: Example DiscoVista visualizations of gene tree discordance on the 1kp dataset (Wickett et al., 2014). a) Branch quartet frequencies graphs. Bars show the frequencies of observing the three quartet topologies around focal branches of the ASTRAL species tree among the main trimmed gene trees of the 1kp dataset. Each internal branch has four neighboring branches, which can be arranged in three ways. The frequency of the species tree topology among gene trees is shown in red, and the other two alternative topologies are shown in blue. The dotted lines indicate the 1/3 threshold. The title of each box indicates the label of the corresponding branch on the associated cartoon tree (also generated by DiscoVista). On the x-axis the exact definition of each quartet topology is shown using the neighboring branch labels separated by “#”. b) Gene tree compatibility graphs. Gene tree analysis on the main model condition of 1kp dataset (FNA2AA-filterlen33), showing the number of genes that highly/weakly support or reject each split. The graph on the left also shows fractions of genes that miss essential components of a clade.

Visualizing the empirical frequency of quartets around each branch can serve two purposes: it gives an interpretable measure of discordance specific to that branch (Sayyari and Mirarab, 2016), and one can check whether the assumptions of ILS are met. If the discordance is purely due to ILS, then one would expect the second and the third hypotheses to have similar frequencies (close to $q$). Lack of this pattern can have many causes, including hybridization (Solís-Lemus et al., 2016). Finally, note that if the length of a species tree branch in coalescent units (Allman et al., 2011) is $d$, then for quartets around it, $p = 1 - \frac{2}{d+2}$, and thus, the quartet frequencies also convey information about the branch length. In the limit, for $d = 0$ (e.g., a polytomy) one would expect all three frequencies to be close to $\frac{1}{3}$ and $p = q = \frac{1}{3}$ can be tested as a null hypothesis (Sayyari and Mirarab, 2017).
In this visualization, for every focal split, a bar graph shows the quartet frequencies around that branch (e.g., Fig. 1a). Moreover, a cartoon tree is generated where leaves are the large groups of taxa collapsed into single leaves and branches are labeled consistently with the bar graph. Inputs to this analysis are a set of gene trees, a species tree, an annotation file that maps each species to a named group and thus defines leaves of the cartoon tree and by extension, the focal splits.

iv) Occupancy analysis (e.g., Figs. 2c and S7cd): Missing data is common in phylogenomics, and common filtering practices can further increase the amount of missing data with potential impact on downstream analyses (e.g., Hosner et al. 2015). DiscoVista can visualize the taxon occupancy for individual species or for groups of taxa; taxon occupancy is defined as the fraction of gene alignments that have at least one of the species from a group. The inputs to this analysis are a set of sequence alignment files (FASTA format), and a species annotation file; the output is a line plot of the occupancy per species (Fig. S7c) or per group (Fig. S7d). DiscoVista can also visualize taxon occupancy as a heatmap (Fig. 2c) that shows the presence/absence of species in gene alignments, and for those present, uses color shades to indicate their non-gapped sequence length compared to the other sequences of the same gene.

v) GC content (e.g., Figs. S7ab): Commonly used models of sequence evolution are stationary and assume that all species have identical base composition. This assumption is often violated in gene coding sequences, especially in the third codon position (Jeffroy et al. 2006). While non-stationary models (Boussa and Gouy 2006) and tests of divergence from stationarity (Ababneh et al. 2006) exist, simply visualizing GC content of different codon position for different extant taxa can help in judging non-stationarity and in deciding whether a GTR analysis is appropriate for some or all of the data (Jarvis et al. 2014; Wickett et al. 2014). DiscoVista generates box plots (distributions) or dot plots (averages) of GC content of each codon position, as well as all three codon positions, for each species. The input to this analysis is a set of gene coding sequences in FASTA format.

3 Results and Discussion

The position of Xenacoelomorpha among deep branches of the Metazoan phylogeny has proved challenging to resolve, with two prevailing hypotheses. One hypothesis puts Xenacoelomorpha as sister to all other Bilateria, while the other hypothesis puts them inside Deuterostomia, implying a dramatic loss of complexity. Intriguingly, these marine worms are bilaterally symmetrical but lack several other features compared to most other bilaterians.

Two independent studies by Rouse et al. (2016) and Cannon et al. (2016) (published simultaneously by Nature) have focused on the position of Xenacoelomorpha in the tree of life. These two studies used different (but overlapping) set of species and each analysis used several reconstruction methods. The two papers come to the same conclusion, putting Xenacoelomorpha as the sister to all other Bilateria. The final results of Cannon et al. (2016) is based on 78 species and 212 orthologous genes with average per taxon occupancy of 80%. Their paper includes alternative analyses based on several subsets of taxa and reconstruction methods. The dataset by Rouse et al. (2016) includes 26 species and 1178 genes (including four Xenoturbella species) with average gene occupancy of 70%. They also report alternative trees using concatenation and ASTRAL (run on 393 genes with 80% occupancy).

Although these two datasets used different set of taxa, by focusing on focal splits, DiscoVista can generate visual comparisons of results across both datasets (Fig. 2). Several interesting patterns emerge. i) The two datasets produce largely congruent results, with only minor differences (Fig. 2). ii) In the Rouse et al. (2016) dataset, aggressive filtering of genes based on their occupancy negatively impacts concatenation trees in terms of BS support and the recovery of some clades (e.g., Chordata). iii) Consistent with literature (Mirarab et al. 2016), ASTRAL run on maximum likelihood gene trees (bestML) seems more accurate than ASTRAL run on the bootstrap replicates (MLBS) gene trees (impacting the recovery of Chordata).

Gene tree discordance figures reveal yet more interesting patterns. Although ASTRAL species trees on maximum likelihood gene trees are mostly congruent in both datasets and with concatenation results, gene trees show high amounts of discordance, and none of the major splits are recovered in most gene trees (Fig. 2l). However, for all of the focal splits, the majority of the gene trees are compatible with the species tree after contracting low support branches (below 75%). Interestingly, maximum likelihood gene trees in Rouse et al. (2016) show somewhat less discordance with major species tree splits compared to those in Cannon et al. (2016). The portion of quartets around three focal branches of the ASTRAL species trees are also interesting (Fig. 2l). For the controversial splits, Nephrozoa (branch labeled as “8”) and Deuterostomia (labeled as “9”), the relative frequency of main topologies are extremely close to
Figure 2: DiscoVista analyses on two Xenoturbella datasets a) An example split definition file for the two Xenoturbella datasets; several lines on top define base splits and are left blank here, but see the full files in Figures S1 and S2 b) DiscoVista Species tree analysis. Rows correspond to focal splits, and columns correspond to alternative species trees reported in two papers [Rouse et al., 2016; Cannon et al., 2016]. The spectrum of blue-green indicates support values for splits compatible with a tree. Note that support values are of different types (Bayesian posterior, concatenation bootstrap support, and multi-locus bootstrap support) and thus may not be directly comparable. Weakly rejected splits correspond to splits that are not present in the tree, but are compatible if low support branches (below 90%) are contracted. In [Rouse et al., 2016], RAxML70_1178, RAxML80_393, and RAxML90 are results of concatenation analyses on genes with average occupancy of 70%, 80%, and 90% respectively. For [Cannon et al., 2016], RAxML212, RAxML212_Acoelomorpha, RAxML336, and RAxML881 are concatenation analyses on 212 orthologous genes of 78 species, 212 orthologous genes after removing Acoelomorpha, 336 orthologous genes on 56 selected species, and 881 orthologous genes on 77 species, respectively. The ASTRAL_212 is the species tree estimated using ASTRAL and 212 orthologous genes of 78 species. c) The occupancy map of 393 gene alignments of [Rouse et al., 2016] with average occupancy of 80%. The spectrum of green color shows the gene length, and the white color indicates missing data. d) Gene tree analysis. This figure shows the portion of RAxML genes for which focal splits (x-axis) are highly (weakly) supported or rejected. Weakly rejected splits are those that are not in the tree but are compatible if low support branches (below 75%) are contracted. e) Frequency of three topologies around focal internal branches of ASTRAL species trees in both datasets. Main topology is shown in red, and the other two alternative topologies are shown in blue. The dotted line indicates the 1/3 threshold. The title of each subfigure indicates the label of the corresponding branch on the tree on the right (also generated by DiscoVista). Each internal branch has four neighboring branches which could be used to represent quartet topologies. On the x-axis the exact definition of each quartet topology is shown using the neighboring branch labels separated by “#”. 

| Code | Name                  |
|------|-----------------------|
| A    | Amphimedon            |
| B    | Strongylocentrotus    |
| C    | Lachnostoma          |
| D    | Bostrychocinerea      |
| E    | Protoschizochordalis |
| F    | Ciona                 |
| G    | Strongylocentrotus    |
| H    | Strongylocentrotus    |
| I    | Strongylocentrotus    |
| J    | Strongylocentrotus    |
| K    | Strongylocentrotus    |
| L    | Strongylocentrotus    |
| M    | Strongylocentrotus    |
| N    | Strongylocentrotus    |
| O    | Strongylocentrotus    |
| P    | Strongylocentrotus    |
| Q    | Strongylocentrotus    |
| R    | Strongylocentrotus    |
| S    | Strongylocentrotus    |
| T    | Strongylocentrotus    |
| U    | Strongylocentrotus    |
| V    | Strongylocentrotus    |
| W    | Strongylocentrotus    |
| X    | Strongylocentrotus    |
| Y    | Strongylocentrotus    |
| Z    | Strongylocentrotus    |
1/3 in both studies. This high level of gene tree discordance around these two branches is likely caused by a combination of high true discordance and also gene tree estimation error; irrespective of which is more prevalent, the high discordance reveals the cause of difficulties faced in resolving these relationships. Interestingly, the portion of quartets for alternative topologies in Rouse et al. (2016) follow the expectations of the MSC theory quite well (i.e., second and third topologies have very close frequencies); the same cannot necessarily be said of the Cannon et al. (2016) gene trees.

To summarize, DiscoVista provides a useful tool for visualizing several patterns of discordance, missing data, and model misspecification in phylogenomic datasets.

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Supplementary Material for “DiscoVista: interpretable visualizations of gene tree discordance”

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This document provides supplementary tables and figures used in the main paper.

## Supplementary Figures

Figures S1 to S7 are all for the plant dataset of [Wickett et al., 2014](#).

![Clade Name | Clade Definition | Section Letter | Components | Show](#)
| Clade Name | Clade Definition | Section Letter | Components | Show |
|-----------|----------------|----------------|------------|------|
| Ambulacraria | All-Bilateria | None | None | 0 |
| Ecdysozoa | Entoprocta | None | None | 0 |
| Xenoturbella | 'Loxosoma pectinaricola'' | None | None | 0 |
| Acoelomorpha | 'Hofstenia miamia'' | None | None | 0 |
| Spiralia | 'Capitella teleta+'+''Lottia gigantea''+''Baseodiscus unicoric''+''Mnemiopsis leidyi''+''Drosophila melanogaster''+''Trichoplax adhaerens''+''Saccoglossus kowalevskii''+''Nematostella vectensis''+''Anneissia japonica''+''Peripatopsis capensis''+''Hofstenia miamia''+''Xenoturbella bocki'' | None | None | 1 |
| Bilateria | 'Gnathostomula paradoxa'+''Schmidtea mediterranea'' | None | None | 0 |
| deuterostomia | 'Hofstenia miamia'+''Xenoturbella profundus'' | None | None | 0 |
| Protostomia | 'Lottia gigantea'+''Ciona intestinalis''+''Gallus gallus''+''Homo sapiens'' | None | None | 0 |
| Deuterostomia | 'Gnathostomula paradoxa'+''Schmidtea mediterranea''+''Hofstenia miamia'' | None | None | 0 |
| All | 'Branchiostoma floridiae'+''Ciona intestinalis''+''Gallus gallus''+''Homo sapiens'' | None | None | 0 |
| Chordata | 'Hofstenia miamia'+''Xenoturbella profundus'' | None | None | 0 |
| Spiralia | 'Lottia gigantea'+''Ciona intestinalis'' | None | None | 1 |
| Ecdysozoa | 'Branchiostoma floridiae'+''Ciona intestinalis''+''Gallus gallus''+''Homo sapiens'' | None | None | 1 |
| Acoelomorpha | 'Branchiostoma floridiae'+''Ciona intestinalis''+''Gallus gallus''+''Homo sapiens'' | None | None | 1 |
| Ecdysozoa | 'Branchiostoma floridiae'+''Ciona intestinalis''+''Gallus gallus''+''Homo sapiens'' | None | None | 1 |
| Ambulacraria | 'Branchiostoma floridiae'+''Ciona intestinalis''+''Gallus gallus''+''Homo sapiens'' | None | None | 1 |
| Clade Name | Clade Definition | Section Letter | Components | Show |
|-----------|----------------|----------------|------------|------|
| Protostomia | Spiralia+Ecdysozoa | None | Spiralia+Ecdysozoa | 1 |
| Deuterostomia | Ambulacraria+Chordata | None | None | 1 |
| Bilateria | Nephrozoa | None | Nephrozoa | 1 |
| Bilateria | Nephrozoa | None | Nephrozoa | 1 |
| Bilateria | Nephrozoa+Xenacoelomorpha | None | None | 1 |
| Xenambulacraria | Ambulacraria+Xenacoelomorpha | None | None | 1 |
| C2 | Chordata+Xenambulacraria(C1) | None | None | 1 |
| C2 | Chordata+Xenambulacraria(C1) | None | None | 1 |
| D2 | Xenacoelomorpha+Deuterostomia | None | None | 1 |
| Bilateria(D3) | D2+Protostomia | None | None | 0 |
| Bilateria(E1) | Xenoturbella+Ambulacraria | None | None | 1 |
| E2 | Chordata+Xenambulacraria(E1) | None | None | 1 |
| E3 | Protostomia+E2 | None | None | 1 |
| Outgroup | All-Bilateria | None | None | 0 |

**Figure S1:** Complete split definitions for the Xenoturbella dataset of [Rouse et al., 2016](#).
| Clade Name | Clade Definition | Section Letter | Components | Show |
|------------|-----------------|----------------|------------|------|
| Xenoturbella | *Xenoturbella bocki* | None | None | 0 |
| Acoelomorpha | Doliolum pharus, *Doliolum pharus mariae* | None | None | 0 |
| Ambulacraria | *Echinodermata* | None | None | 1 |
| Entoprocta | *Loosenia* | None | None | 0 |
| Ecdysozoa | *Prasoplopus* | None | None | 1 |
| Chordata | *Branchiostoma* | None | None | 1 |
| Spiralia | *Crassostrea giga* | None | None | 1 |
| Xenacoelomorpha | *Xenoturbella bocki* | None | None | 1 |
| Deuterostomia | *Protostomia* | None | None | 1 |
| All | *Clione varius* | None | None | 0 |
| Protostomia | *Spiralia-Ecdysozoa* | None | None | 1 |

**Figure S2:** Complete split definitions for the Xenoturbella dataset of Cannon et al. (2016).
Figure S3: DiscoVista Specie tree analysis on plants dataset: Rows correspond to major orders and clades, and columns correspond to the results of different methods reported in two different closely related datasets. The spectrum of blue-green indicates amount of MLBS values for monophyletic clades. Weakly rejected clades correspond to clades that are not present in the tree, but are compatible if low support branches (below 90%) are contracted.
Figure S4: DiscoVista Specie tree analysis on plants dataset: Rows correspond to major orders and clades, and columns correspond to the results of different methods reported in two different closely related datasets. Weakly rejected clades correspond to clades that are not present in the tree, but are compatible if low support branches (below 90%) are contracted.
Figure S5: Gene tree analysis on plants dataset: The portion of RAxML genes for which important clades (x-axis) are highly (weakly) supported or rejected for three model conditions of the plants dataset. FAA-filterlen33: gene trees on amino acids sequences, and fragmentary sequences removed (66% gaps or more) FNA2AA-f25: amino acid sequences back translated to DNA, and sequences on long branches (25X median branch length) removed; FNA2AA-filterlen33: amino acid sequences back translated to DNA, and fragmentary sequences removed (66% gaps or more). Weakly rejected clades are those that are not in the tree but are compatible if low support branches (below 75%) are contracted.
Figure S6: Gene tree analysis on plants dataset: The number of RAxML genes for which important clades (x-axis) are highly (weakly) supported or rejected or are missing of three model conditions (same as S5) of the plants dataset. Weakly rejected clades are those that are not in the tree but are compatible if low support branches (below 75%) are contracted.
Figure S7: DiscoVista analyses on 1kp dataset a,b) GC content analysis of the 1kp dataset using boxplots and dot plot respectively for first, second, third, as well as all three codon positions. In dot-plot each dot shows the average GC content ratio for each species in all (red), first (pink), second (light blue), and third (dark blue) codon positions. c) occupancy analysis on the 1kp dataset over each individual species for two model conditions. FNA2AA-f25: amino acid sequences back translated to DNA, and sequences on long branches (25X median branch length) removed; FNA2AA-filterlen33: amino acids sequences back translated to DNA, and fragmentary sequences removed (66% gaps or more). d) occupancy analysis of major clades in the 1kp dataset and the same model conditions as (b).
Structure of parameter files

**splits definitions:** The splits definition file has 7 columns separated with tabs. In the first column split names are listed, in the second column the species or other splits that define this split are listed separated with “+” or “-” signs. “+” signs are used to add splits and species, and “-” signs to subtract species or splits. In the third column (you might leave this column blank) a name can be defined that is used to specify the part of the tree that the splits belongs to, e.g. 1-Base, or Base. Forth column defines splits components where in the absence of any of them splits is considered as missing. The fifth column indicates whether the split should be shown in the species tree (gene trees) analysis. The sixth column is used to define components from the other side of the split, where in the absence of any of them the split is considered as missing. Note that if you leave this column blank, “All” minus this split will be considered as the other side component. The last column is used to add any comments to this file. Also note that, a split with the name “All” should be defined which consists of all the species names in your analysis.

**Annotation file:** In this file, there should be a row for each species. The first column of the annotation file specifies the name of species, and the second column defines the split that this species belongs to. Columns are separated by tab.

**Installation, Commands, and Usage**

In order to install DiscoVista from source please refer to [https://github.com/esayyari/DiscoVista](https://github.com/esayyari/DiscoVista). DiscoVista relies on different R, python, some C packages, as well as ASTRAL. In order to make the installation easier we also provide a docker image available at [https://hub.docker.com/r/esayyari/discovista/](https://hub.docker.com/r/esayyari/discovista/). In order to use this image, after installing docker, it is sufficient to download it using the following command:

```
docker pull esayyari/discovista
```

Now, you would run different analyses using the following command:

```
docker run -v <absolute path to data folder>:/data esayyari/discovista discoVista.py [OPTIONS]
```

**Species discordance analysis:** For this analysis you need the path to the species trees folder, the clade definitions, the output folder, and a threshold. For more information regarding the structure of the species tree folder please refer to [https://github.com/esayyari/DiscoVista](https://github.com/esayyari/DiscoVista). Then you would use the following command using docker:

```
docker run -v <absolute path to data folder>:/data esayyari/discovista discoVista.py -m 0 -p <path to species trees folder> -t <threshold> -c <path to split definition> -o <output folder> -y <model condition ordering file> -w <splits ordering file>
```

**Discordance analysis on gene trees:** For this analysis you need the path to the gene trees folder, the clade definitions, the output folder, and a threshold. For more information regarding the structure of the gene trees folder please refer to [https://github.com/esayyari/DiscoVista](https://github.com/esayyari/DiscoVista). Then you would use the following command with docker:

```
docker run -v <absolute path to data folder>:/data esayyari/discovista discoVista.py -m 1 -p <path to gene trees folder> -t <threshold> -c <path to split definition> -o <output folder> -w <splits ordering file>
```

**GC content analysis:** For this analysis you need the path to the coding alignments (in FASTA format) with the structure described in more details at [https://github.com/esayyari/DiscoVista](https://github.com/esayyari/DiscoVista) and the output folder. You would use the following command in docker:

```
docker run -v <absolute path to data folder>:/data esayyari/discovista discoVista.py -m 2 -p <path to alignments folder> -o <output folder>
```
**occupancy analysis:** For this analysis you need the path to the alignments (in FASTA format) with the structure described in more details at https://github.com/esayyari/DiscoVista, and the output folder. Then you can use this command with the docker:

```bash
docker run -v <absolute path to data folder>:/data esayyari/discovista discoVista.py -p $path -m 3 -a <path to annotation file> -o <output folder>
```

**Relative frequency analysis:** For this analysis you need the path to the gene trees and species tree folder (structure described on the Github), annotation file, outgroup clade name, output folder. Using docker you would do this analysis with the following command:

```bash
docker run -v <absolute path to data folder>:/data esayyari/discovista discoVista.py -p $path -m 5 -a <path to annotation file> -o <output folder>
```

If desired, the cartoon tree can be shown rooted, requiring the name of the outgroup as input (e.g. Base or Outgroup).

```bash
docker run -v <absolute path to data folder>:/data esayyari/discovista discoVista.py -p $path -m 5 -a <path to annotation file> -o <output folder> -g <outgroup clade name>
```