

# AliTV – interactive visualization of whole genome comparisons

Markus J. Ankenbrand<sup>1,\*</sup>, Sonja Hohlfeld<sup>1,2,\*</sup>, Thomas Hackl<sup>2,3</sup>, and Frank Förster<sup>2,4</sup>

<sup>1</sup>Department of Animal Ecology and Tropical Biology, Julius Maximilian University, Würzburg, Germany

<sup>2</sup>Department of Bioinformatics, Julius Maximilian University, Würzburg, Germany

<sup>3</sup>Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

<sup>4</sup>Center for Computational and Theoretical Biology, Julius Maximilian University, Würzburg, Germany

\*These authors contributed equally to this work

## ABSTRACT

Whole genome alignments and comparative analysis are key methods in the quest of unraveling the dynamics of genome evolution. Interactive visualization and exploration of the generated alignments, annotations, and phylogenetic data are important steps in the interpretation of the initial results. Limitations of existing software inspired us to develop our new tool AliTV, which provides interactive visualization of whole genome alignments. AliTV reads multiple whole genome alignments or automatically generates alignments from the provided data. Optional feature annotations and phylogenetic information are supported. The user-friendly, web-browser based and highly customizable interface allows rapid exploration and manipulation of the visualized data as well as the export of publication-ready high-quality figures. AliTV is freely available at <https://github.com/AliTVTeam/AliTV>.

Keywords: Comparative genomics, alignment, visualization

## INTRODUCTION

Advances in short- and long-read sequencing and assembly over the last decade (Salzberg et al., 2011; Chin et al., 2013; Hackl et al., 2014) have made whole genome sequencing a routine task for biologists in various fields. Public sequence databases already contain several thousand of draft and finished genomes (Benson et al., 2013), with many more on the way (Pagani et al., 2012). In particular, high throughput sequencing projects of pathogen strains related to recent outbreaks (Rasko et al., 2011), and large scale ecological studies targeting microbial communities and pan genomes of populations using metagenome and single cell sequencing approaches contribute in this process (Turnbaugh et al., 2007; Kashtan et al., 2014). These rich data sets can be explored for large-scale evolutionary processes using comparative genomics and whole genome alignments, revealing genomic recombinations (Didelot et al., 2012; Namouchi et al., 2012; Yahara et al., 2014), islands and horizontal gene transfer (Avrani et al., 2011; Coleman et al., 2006; Langille et al., 2008) as well as the often related dynamics of mobile or endogenous viral elements (Fischer, 2015; Touchon and Rocha, 2007). Other applications of whole genome comparisons include the analysis of paleopolyploidization events (Vanneste et al., 2014) and quantitative measurements of intra-tumour heterogeneity (Schwarz et al., 2015).

However, to facilitate proper interpretation of the obtained whole genome comparisons, visualization is key. One of the first tools to provide an interactive graphical representation of aligned genomes is the multiple whole genome alignment program Mauve (Darling et al., 2004). Mauve represents genomes in a co-linear layout with homologous syntenic blocks indicated by colors and connecting lines. The interactive stand-alone viewer ACT (Carver et al., 2008), in addition to alignment blocks, supports the representation of genomic annotations, such as genes. The R library genoPlotR (Guy et al., 2010) and the Python based application EasyFig (Sullivan et al., 2011), both also based on a co-linear layout and supporting feature annotations, lack interactive analysis features as they are designed to generate static figures.

In addition to co-linear layouts, tools using circular representations of genomes have been developed. BLASTatlas (Hallin et al., 2008) and BRIG (Alikhan et al., 2011) use multiple concentric rings to represent data of individual genomes, with BRIG also providing an interactive

graphical interface. *GenomeRing* (Herbig et al., 2012) uses a circular representation as well, however, places all genomes on the same ring and syntenic blocks are connected with arcs extending into the center of the ring.

The web-based comparative genomics software *Sybil* (Riley et al., 2012) provides interactive co-linear visualization of multiple whole genome alignments with feature annotations and also supports a phylogenetic tree alongside the alignments. The software builds on a relational Chado database schema and, therefore, requires upload and import of custom data sets prior to analysis.

During our analysis of existing software, we found that interactive tools are useful for data exploration, but offer limited support for the figure export and at low qualities. Scripting-based tools provide higher levels of customization and figure quality, however, require familiarity with the respective language, thus often rendering the generation of figures time-consuming. For web- and database-based suites, such as *Sybil*, the upload and import procedure complicate utilization and limit applicability.

Here we present our stand-alone application *AliTV* (Alignment Toolbox and visualization) designed for interactive visualization of multiple whole genome alignments. *AliTV* aims to enable researches to either directly read or automatically generate new whole genome alignments, rapidly explore the results, manipulate and customize the visualization and, at the end of the day, export appealing, publication-grade figures. *AliTV* reads sequence and annotation or alignment data in common formats (FASTA, GenBank, GFF, MAF, Newick, ...), and internally computes alignments using *lastz* (Harris, 2007). The user-friendly interface is built on the state-of-the-art *D3.js* JavaScript framework and can be utilized in a platform independent manner with common web browsers. Genomes are represented in a highly customizable co-linear layout including annotations and an optional phylogenetic tree. The tree is not computed by *AliTV* but has to be provided during data generation. Also the order of genomes is not automatically optimized to minimize rearrangements. Customizations to the figure by the user can be saved, reloaded, and exported to high quality SVG files.

## METHODS

Our tool *AliTV* is divided into two parts. The first non-interactive part is required for the generation of the input files for our interactive viewer. The second part represents that interactive viewer in the form of a SVG file embedded in a HTML5 website. The latest version of our code can be obtained from GitHub (<https://github.com/AliTVTeam/AliTV>). It is planned to adjust *AliTV* in order to integrate it into the *BioJS* registry (<https://biojsnet.herokuapp.com/>, Corpas et al. (2014)). The general design of *AliTV* assures, that *AliTV* runs on different hard- and software platforms, e.g. Linux, MacOSX, and Windows. The following sections describe those parts in more detail.

### Data Preparation

The data preparation is performed by a single Perl script named *alitv.pl*. This script uses a set of different Perl modules to import incoming data and generate valid JSON input data for our visualization engine described in the next paragraph. One of our aims is to support as many different input formats for sequence and annotation information as possible. Therefore, we used the well tested and broadly accepted *BioPerl* as basis for our modules (Stajich et al., 2002).

The script *alitv.pl* uses a YAML file to specify the different input files. Moreover, an easy-to-use-mode is available which requires only a couple of input files and generates the required YAML file on the fly. This generated YAML settings file might be used to reproduce *AliTV* results or can be used as starting point to alter configuration parameters.

During the preparation step, *AliTV* requires all-vs-all alignments of the complete sequence set. Those alignments are generated or user provided. The current version of *alitv.pl* requires *lastz* to generate all alignments in MAF format (Harris, 2007). Nevertheless, *BioPerl* supports a broad range of alignment formats. Therefore, other programs can easily be added to the list of supported alignment programs. Moreover, the ability to use existing alignments allows a huge time benefit, when *AliTV* parameters are changed to optimize the visualization via YAML settings file in a non-interactive manner. Thus future versions of *alitv.pl* will support caching of alignments based on checksums to avoid unnecessary recalculations.

The final result of our *alitv.pl* is a JSON file, which can be load into our interactive visualization page.

**Table 1.** Chloroplast genomes of the parasitic and non-parasitic plants used in the case study.

Species	Accession	Life-style	Reference
<i>Olea europaea</i>	NC_013707	non-parasitic	Messina (2010)
<i>Lindenbergia philippensis</i>	NC_022859	non-parasitic	Wicke et al. (2013)
<i>Cistanche phelypaea</i>	NC_025642	holo-parasitic	Wicke et al. (2013)
<i>Epifagus virginiana</i>	NC_001568	holo-parasitic	Wolfe et al. (1992)
<i>Orobancha gracilis</i>	NC_023464	holo-parasitic	Wicke et al. (2013)
<i>Schwalbea americana</i>	NC_023115	hemi-parasitic	Wicke et al. (2013)
<i>Nicotiana tabacum</i>	NC_001879	non-parasitic	Kunnimalaiyaan and Nielsen (1997)

### Interactive Visualization

AliTV is implemented in JavaScript. Our code is documented using JSDoc 3 (version 3.4.0 <http://usejsdoc.org/>, 02.06.2016). AliTV generates a SVG which is presented within a browser using HTML5. A tutorial is available at <https://alitr.readthedocs.io/en/latest/index.html>.

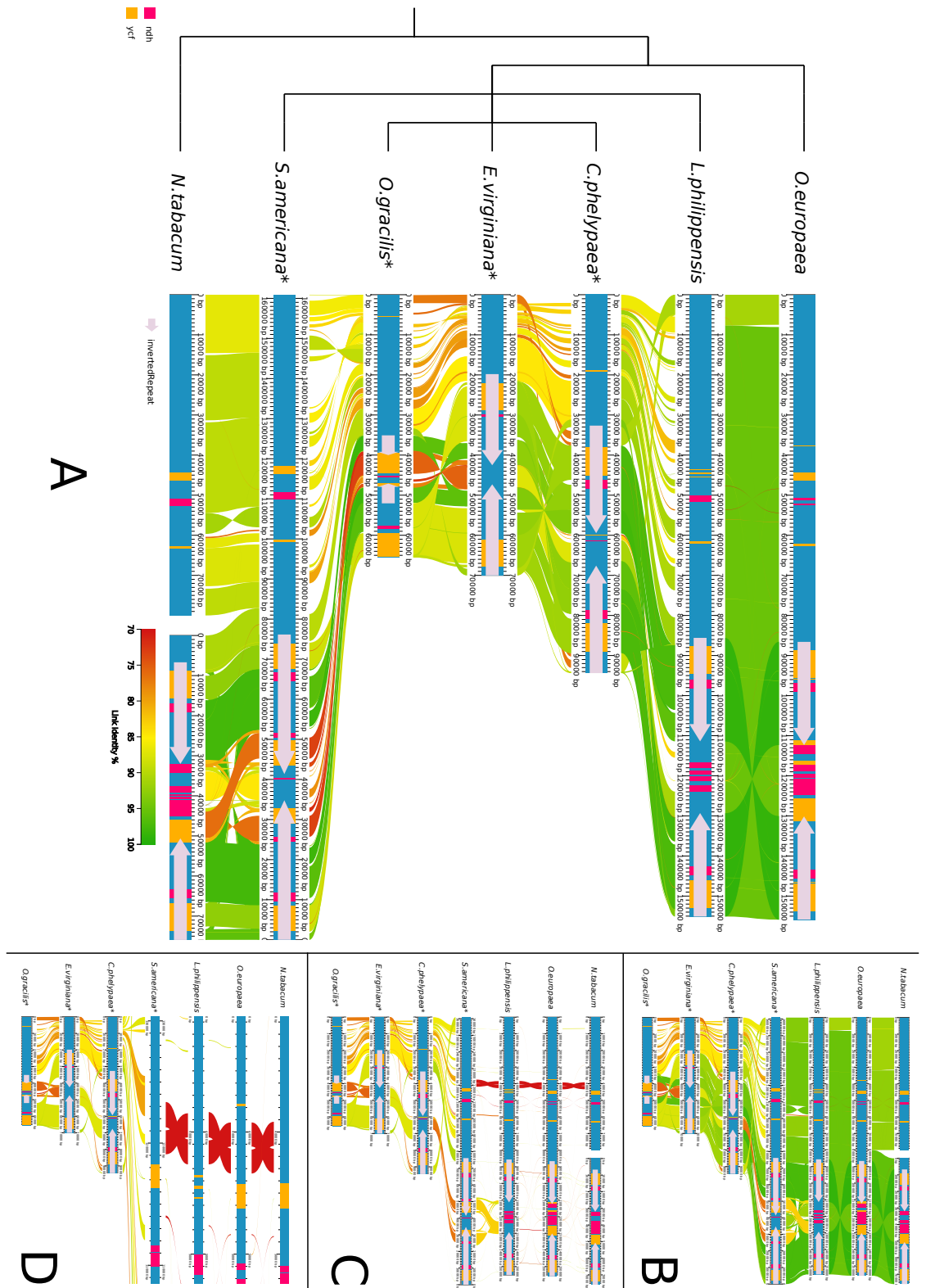
To gain advanced application possibilities we use different libraries. The JavaScript library D3.js 3.5.17 (<http://d3js.org/>, 06.06.2016) provides a wide range of pre-built functions for calculating and drawing the interactive figure. In addition, AliTV employs JQuery 2.2.4 (<https://jquery.com/>, 06.06.2016) to ease access to several parts of the figure. This is helpful for hiding selected sequences, genes or links. JQueryUI 1.11.4 (<https://jqueryui.com/>, 06.06.2016) gives us the possibilities to add user-friendly interactions to AliTV. With sliders the user has the chance to specify values for link length and link identity. Context menus offer direct and native interactions with the figure.

To guarantee correct code functionality we engineer AliTV according to the Test Driven Development. First we write an automated test case that defines a new function. Then we add the minimum amount of code to make the test pass. Finally we refactor the code to accepted standards. We use Jasmine 2.3 (<http://jasmine.github.io/>, 06.06.2016), as framework for testing our JavaScript code. The tests can run either via the SpecRunner or the command line using the taskrunner grunt 1.0.0 (<http://gruntjs.com/>, 06.06.2016).

## RESULTS AND DISCUSSION

To demonstrate the capabilities of AliTV we describe a short case study using seven published chloroplast genomes (table 1). Four of the chloroplasts belong to parasitic plant species and three to non-parasitic ones. Parasitic plants rely much less or not at all on photosynthetic activity, a trait that should be reflected in the genomic structure of their chloroplast genomes. To assess this hypothesis the chloroplast genomes were downloaded from NCBI and processed with `alitr.pl`. For demonstration purposes, the chloroplast genome of *Nicotiana tabacum* was split in two pieces to represent an unfinished genome with more than one contig, and the genome sequence of *Schwalbea americana* was reverse-complemented (flipped). The pair-wise whole genome alignments are visualized by AliTV (figure 1A). The left-hand side of the display panel shows the phylogenetic tree for the seven species with species names as tip labels (parasitic plants are highlighted with an asterisk). The tree has been created provided in accordance to NCBI taxonomy (Sayers et al., 2009). Next to the tip labels, each genome is drawn as a scaled and annotated horizontal bar. The orientation of the *S. americana* genome was swapped back to match the orientation of the other genomes, indicated by the tick coordinates in reverse order (0 on the right side). *N. tabacum* is represented by two bars as the sequence has been split into two parts. On those bars features (e.g. genes or inverted repeats (IRs)) are shown as either rectangles or arrows. Alignments between adjacent genomes are represented as colored ribbons. The bottom legend shows the default color scale from red to green corresponding to low and high identity respectively.

The most striking observation is that three of the chloroplast genomes have drastically reduced sizes. All of those are parasitic (table 1). Interestingly the chloroplast genome size of *S. americana* is similar to that of the non-parasitic plants. This can be explained by the life style of *S. americana* which is hemi-parasitic in contrast to the other parasitic plants which are holo-parasites. The features shown are the IR regions as arrows, the hypothetical chloroplast open reading frames as orange and the genes of the *ndh* family as pink rectangles. First, it can be seen that there is a big variation in size of the inverted repeats. While the IR of *Orobancha gracilis* is the shortest with roughly 5000 bp, that of *S. americana* is the largest with roughly 35000 bp. Second, there are less genes of the *ndh*



family on *Cistanche phelypaea*, *Epifagus virginiana*, *O. gracilis*, and *S. americana*. Members of the *ndh* gene family encode subunits of the NADH dehydrogenase-like complex, which is involved in chlororespiration (Martín and Sabater, 2010). However they are not required for plant growth under optimal conditions (Burrows, 1998). The absence of *ndh* genes in chloroplasts of parasitic plants has been studied in detail in Wicke et al. (2013). Loss of *ndh* genes has also been reported for photosynthetic plants such as some conifers and orchids (Wakasugi et al., 1994; Kim et al., 2015). Looking at the pairwise similarities of adjacent genomes, it is apparent that the non-parasitic plants (e.g. *Olea europaea* and *Lindenbergia philippensis*) have high overall sequence identity. In contrast, the sequence similarity within parasitic plants is lower. This observation can help framing a hypothesis about the evolutionary pressure on chloroplasts of parasitic plants. Another interesting observation is the distribution of missing regions of *C. phelypaea* in comparison to *L. philippensis*. Missing regions are distributed all over the genome and the order of the remaining parts remains stable. Wicke et al. (2013) describe an inversion in the large single copy region of *S. americana* compared to non-parasitic plants which is clearly visible by the link to *N. tabacum* around the 115 kbp position. All these observations can be made by simply looking at the raw figure created by `alitv.pl` and visualized by `AliTV`. However the figure can be analyzed interactively in more detail. One shortcoming of the linear representation of whole genome alignments is the limited comparability of non-adjacent sequences. Therefore, `AliTV` provides a way for the user to re-order the genomes on the figure (figure 1B). If reordering causes inconsistencies with the phylogenetic tree, the tree is hidden and a warning message is displayed. Furthermore, the links can be filtered by their alignment identity. The default setting is to display only links with minimal identity of 70%. But sometimes it might be interesting to look at regions with less similarity. To see these regions it is also important to hide large regions with high similarity. This can be achieved by changing the identity via a slider (figure 1C). After setting the identity range to 50 % to 90 % red 'X'-shaped links between *N. tabacum*, *O. europaea*, *L. philippensis*, and *S. americana* become apparent. For detailed inspection of regions of interest, `AliTV` provides a zoom function (figure 1D). This way the exact location of the alignments can be traced to the locations of *psaA* and *psaB*. Moreover `AliTV` provides functions like alignment length filtering, selective hiding of sequences, links and features, change of orientation (reverse complement) and rotation of circular chromosomes. Finally, it is possible to tweak many graphical parameters, such as colors, labels or spacing, directly via the interface to produce a publication quality figure which can be saved in SVG format. Furthermore, the current state can be saved in JSON format in order to share it with collaborators or continue the work with `AliTV` at a later time.

## CONCLUSION

The case study demonstrates the suitability of `AliTV` as a tool for visualizing and analyzing whole genome comparisons. `AliTV` can be used to easily create a figure that show cases many genomic features at once. Furthermore the rich interactive features enable the exploratory analysis and discovery of previously unknown features. Thus novel hypotheses can be generated that can then be validated with experimental methods. So `AliTV` is a useful tool that will help scientists to find biologically meaningful information in the vast amount of genomic data.

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