

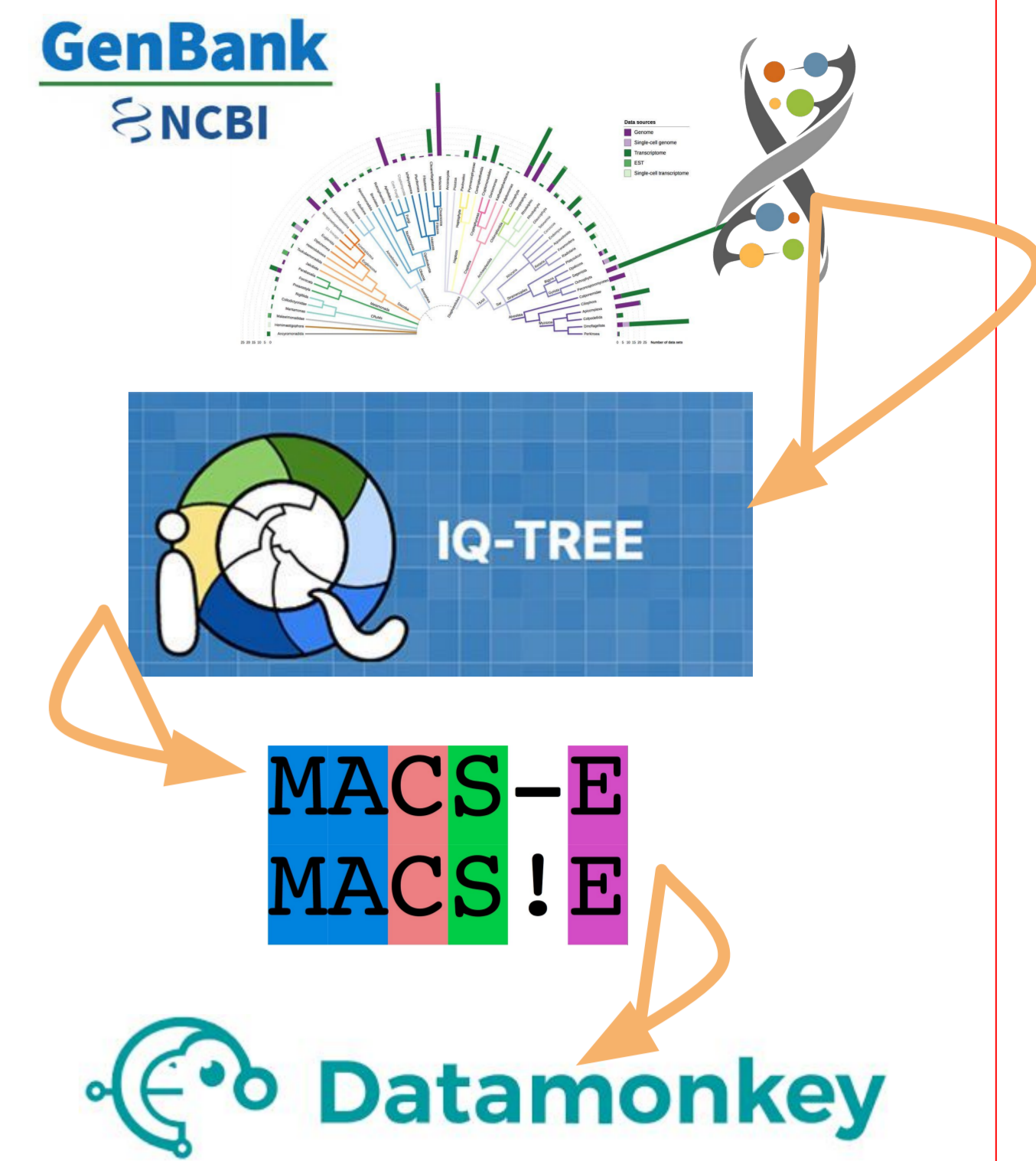
Evolution of photolyases in Kinetoplastid protists

Hypothesis

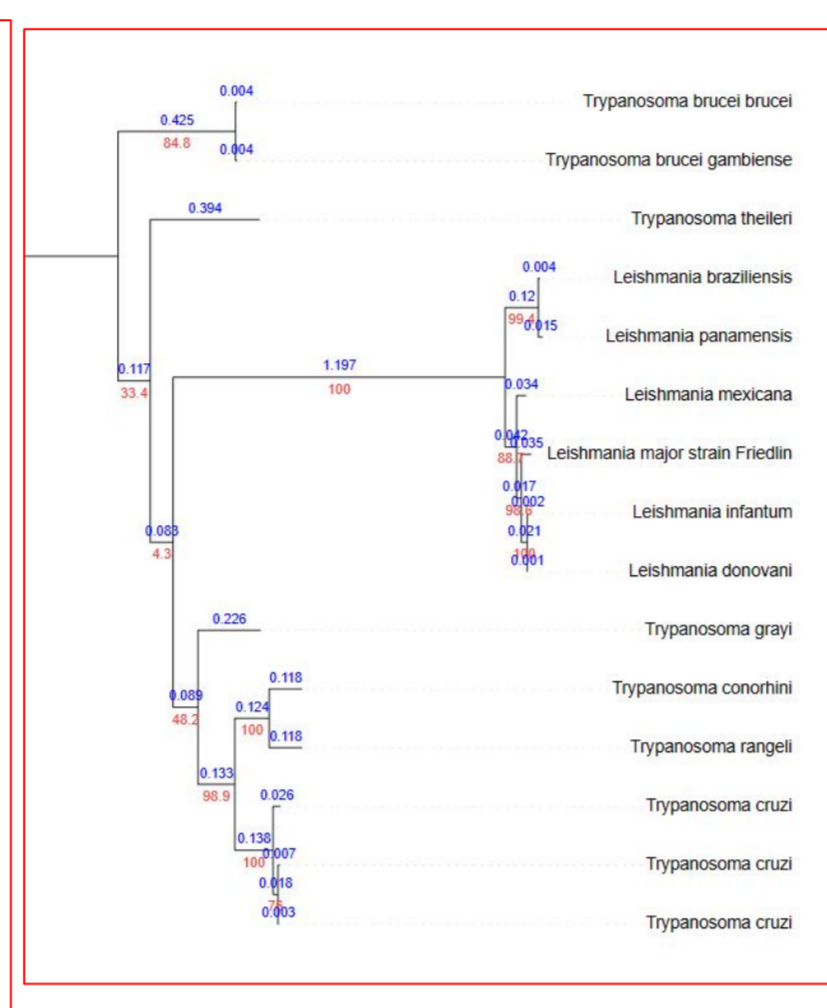
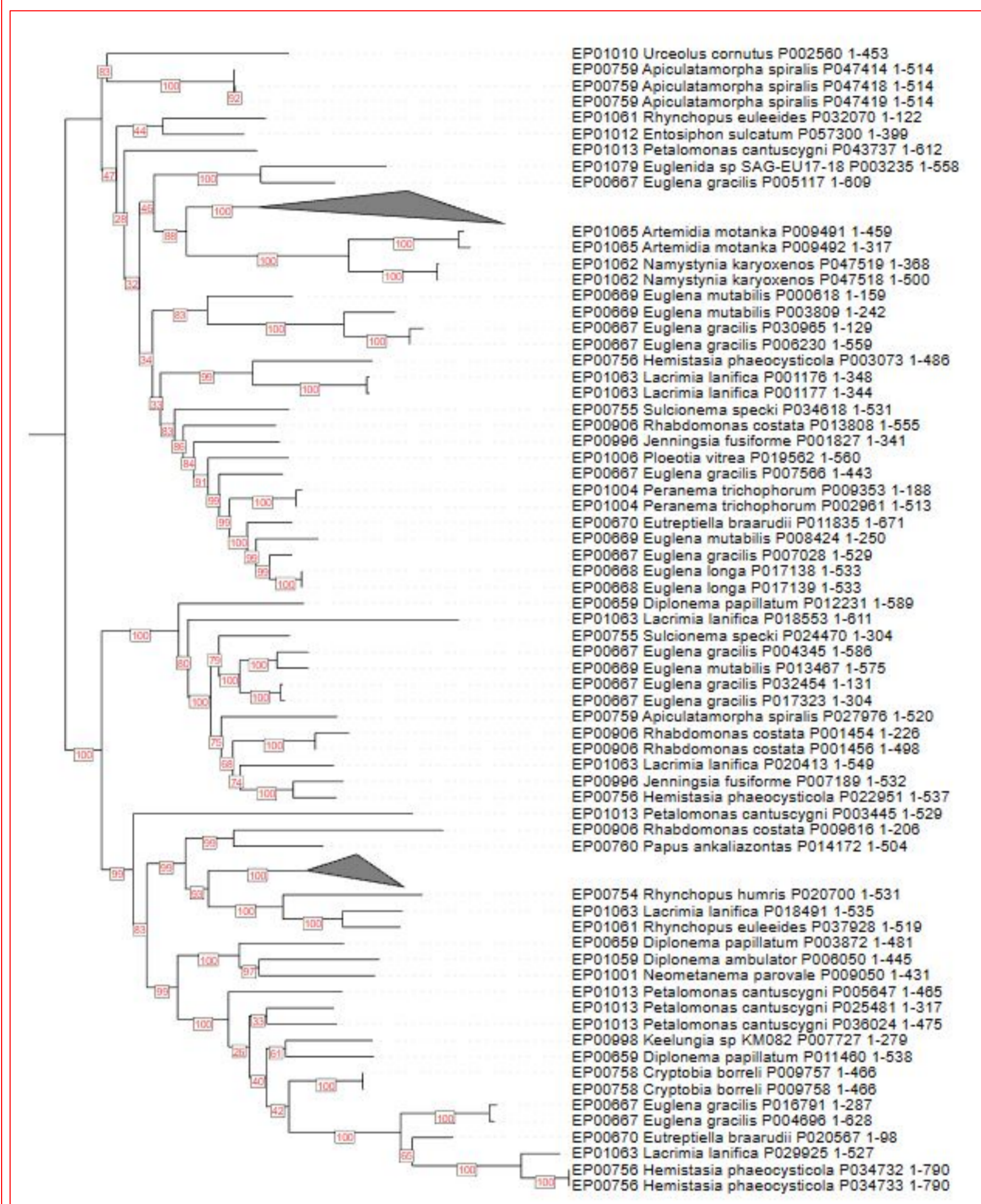
We hypothesize that some of the fascinating abilities of Kinetoplastida, including their unique mtDNA organisation and RNA modification mechanisms, can be explained by an evolutionary scenario that involves UV as a strong selection factor. That predicts positive selection being present on the photolyase sequences, which can be tested using bioinformatic algorithms.

Methods

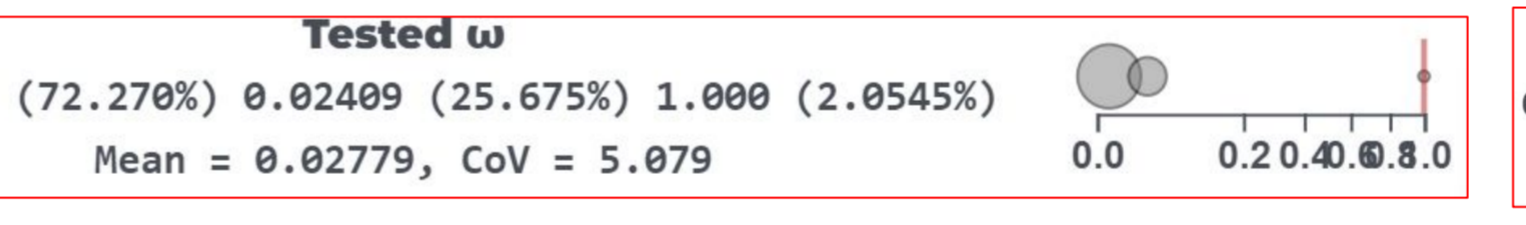
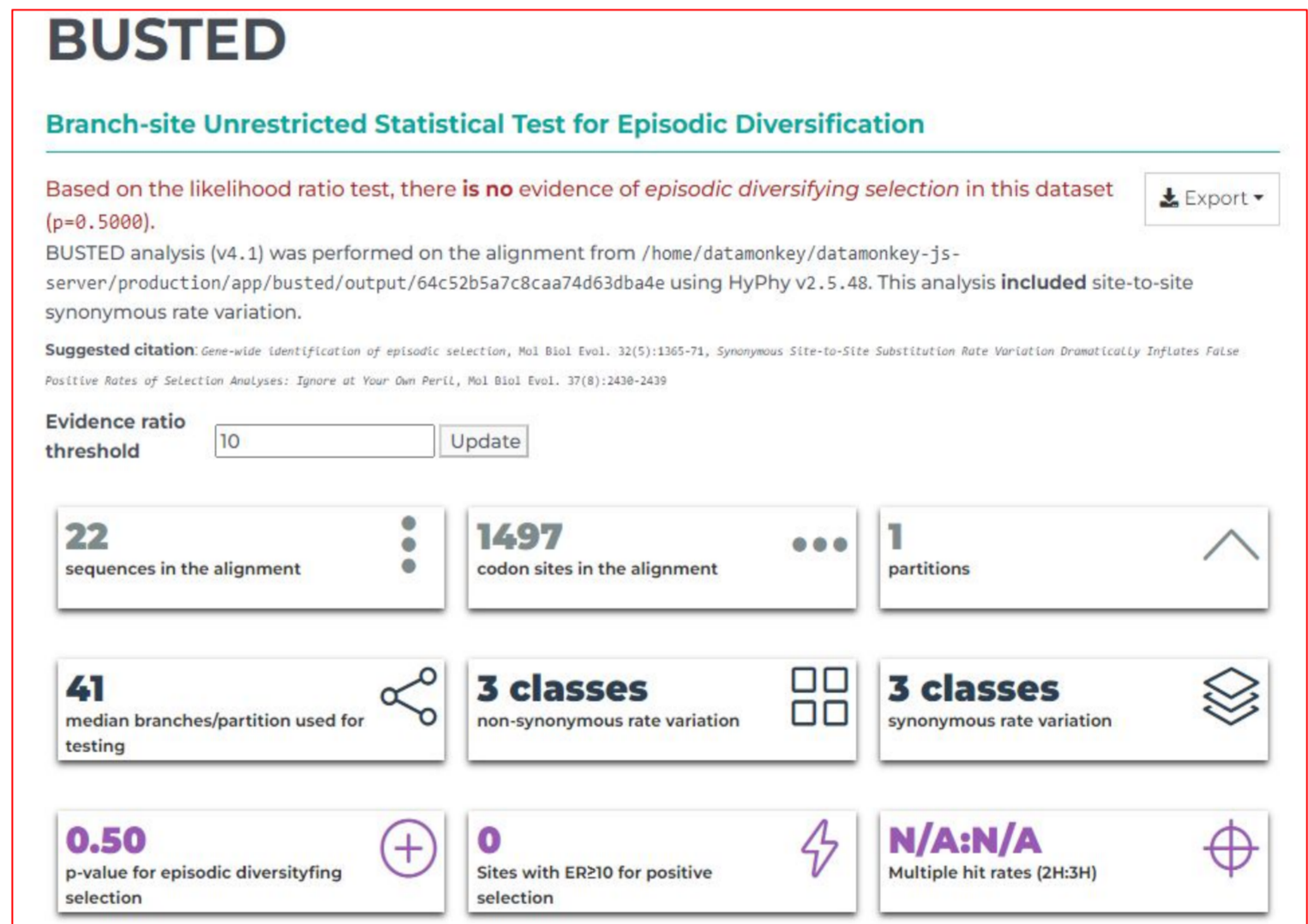
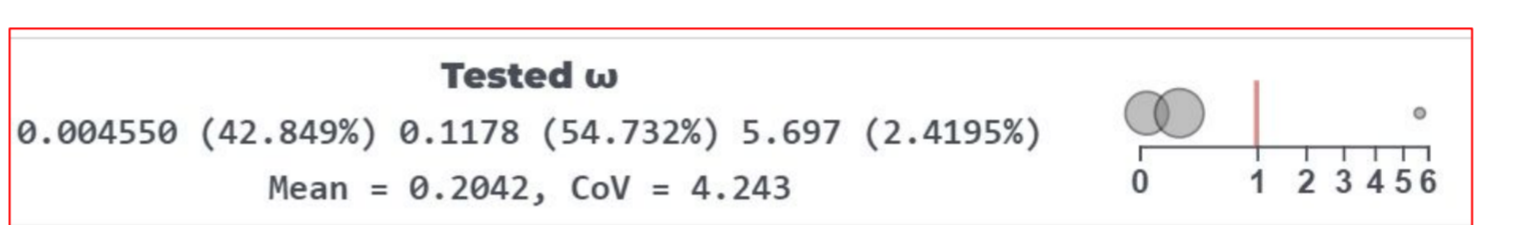
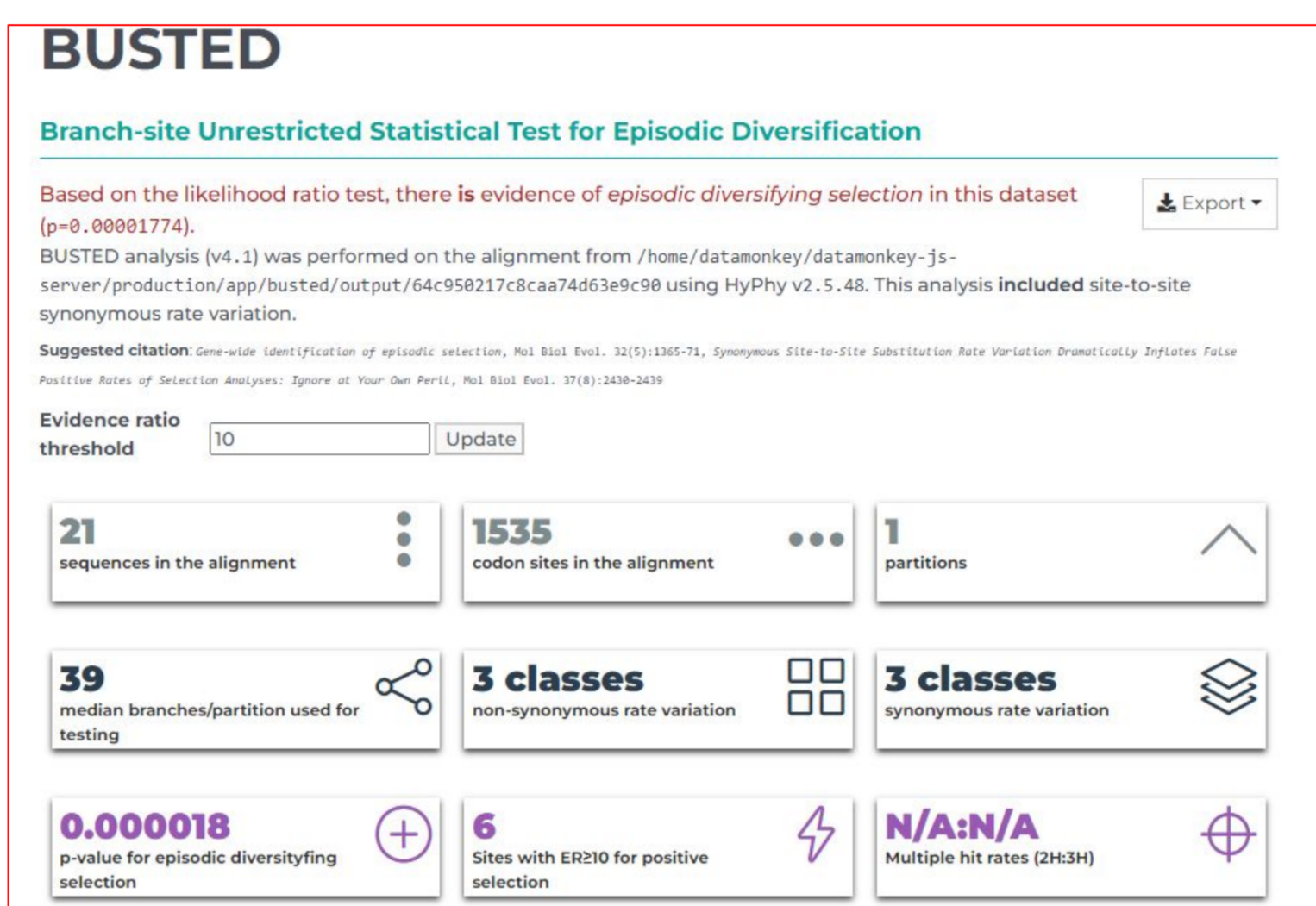
Photolyase-encoding sequences were retrieved from TriTrypDB and EukProt database (for Euglenids), from PhycoCosm (for Chlorophyta). Orthology was verified by building a protein phylogeny. Codon-wise alignment with MACSE was performed for the test set of 23 orthologous photolyase genes from kinetoplastids and control set of 16 photolyase genes from Chlorophytes; alignments were manually trimmed. Dn/ds was calculated using Datamonkey package.



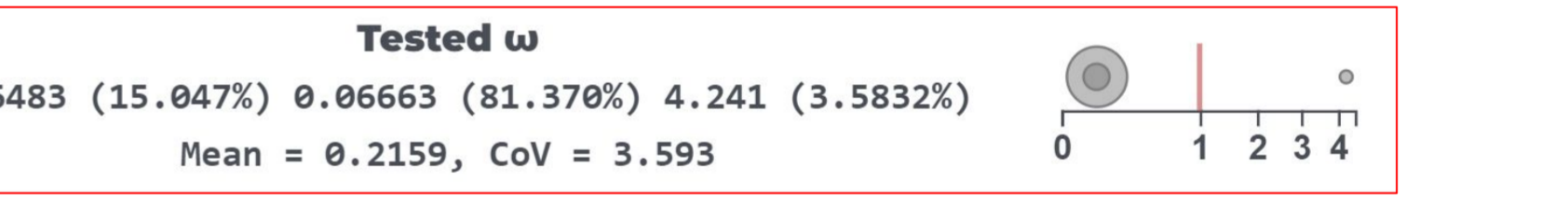
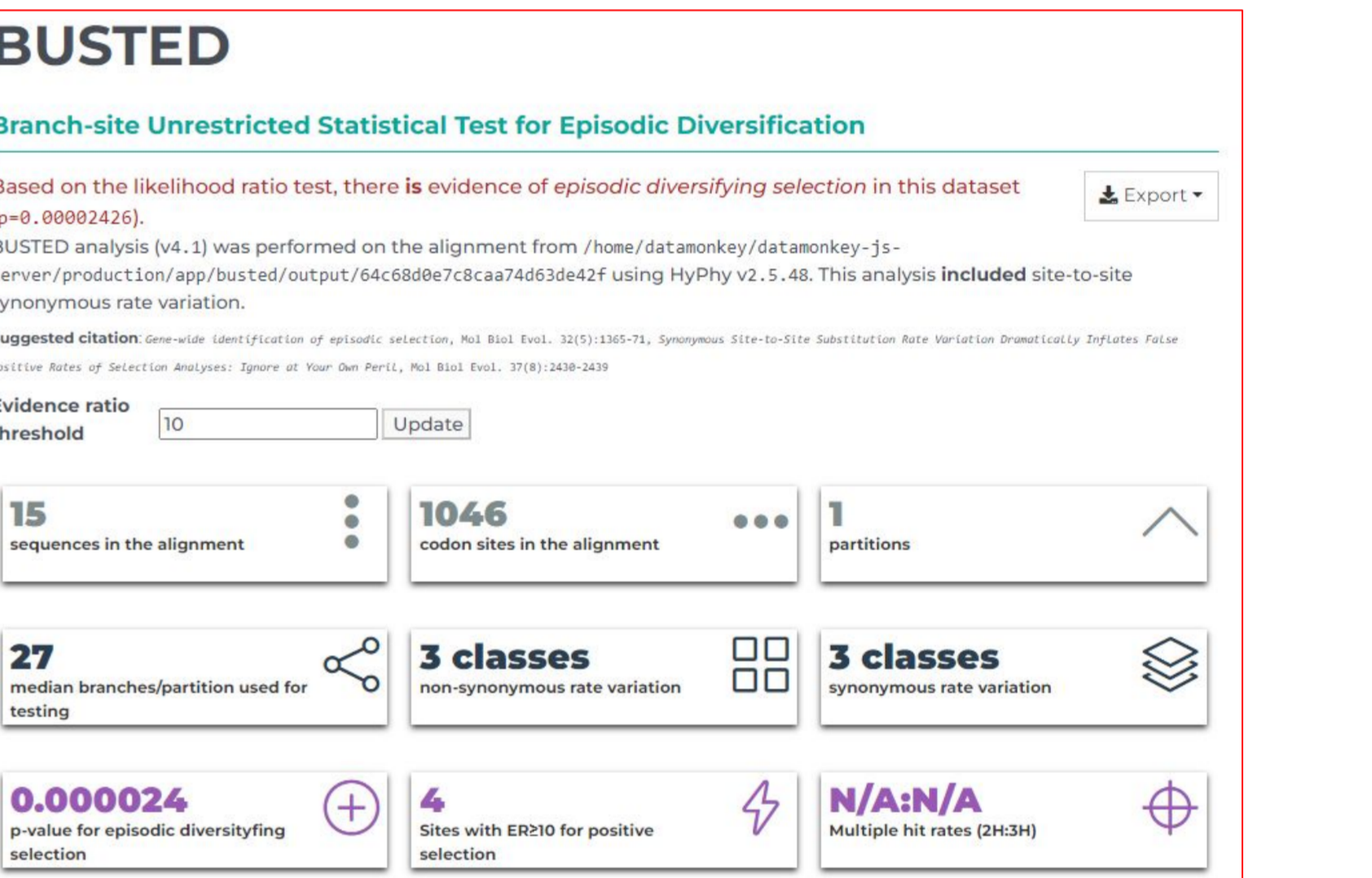
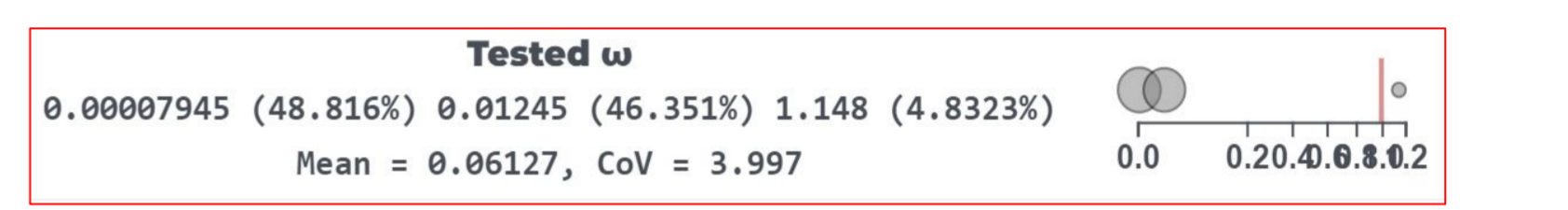
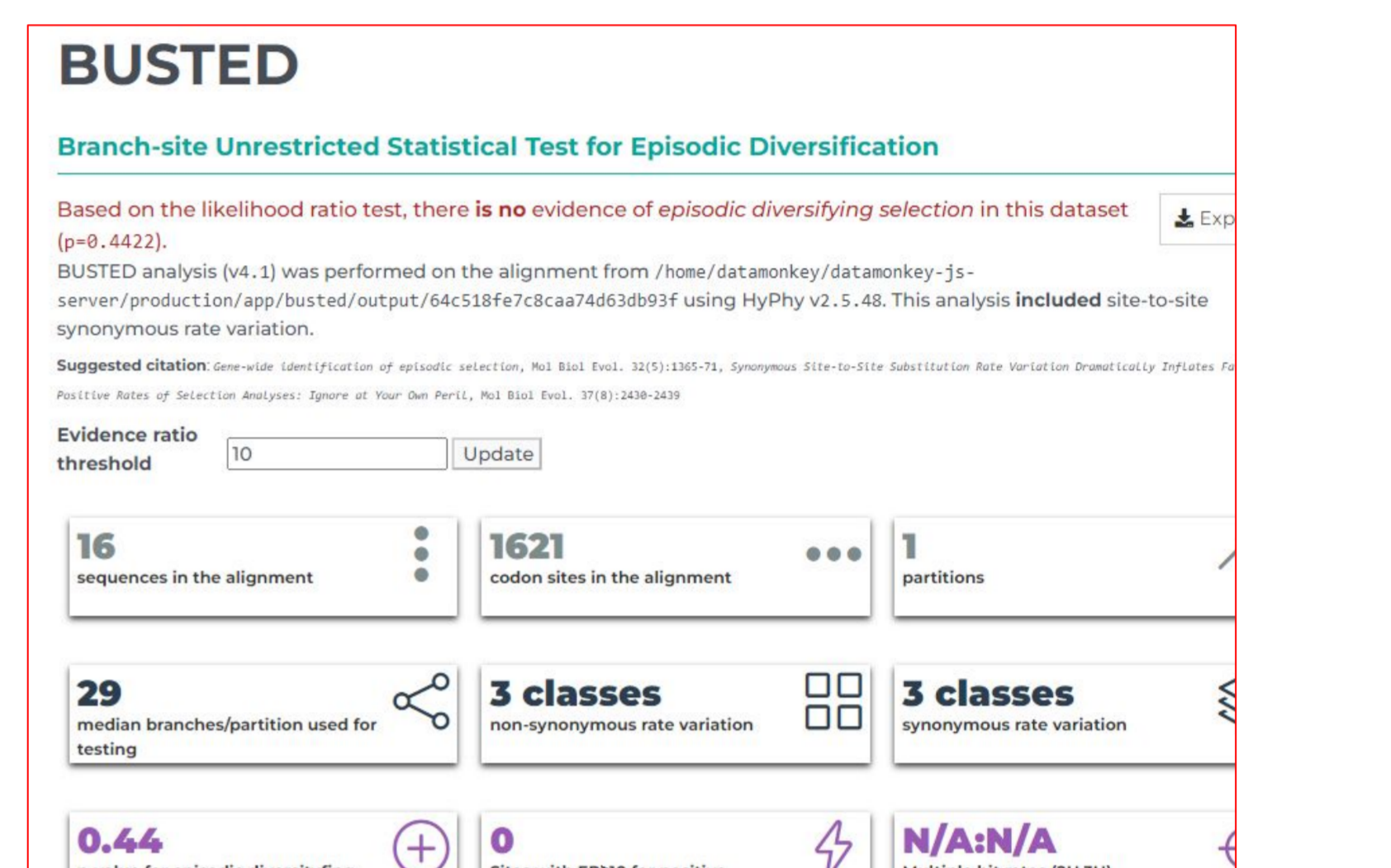
Results & conclusions



Kinetoplastid photolyases dn/ds (test)



Chlorophyte photolyases dn/ds (control)



Datamonkey dn/ds analysis suggests that episodic diversifying selection on photolyases might be present in Kinetoplastids.

Possible experiment principal pipelines

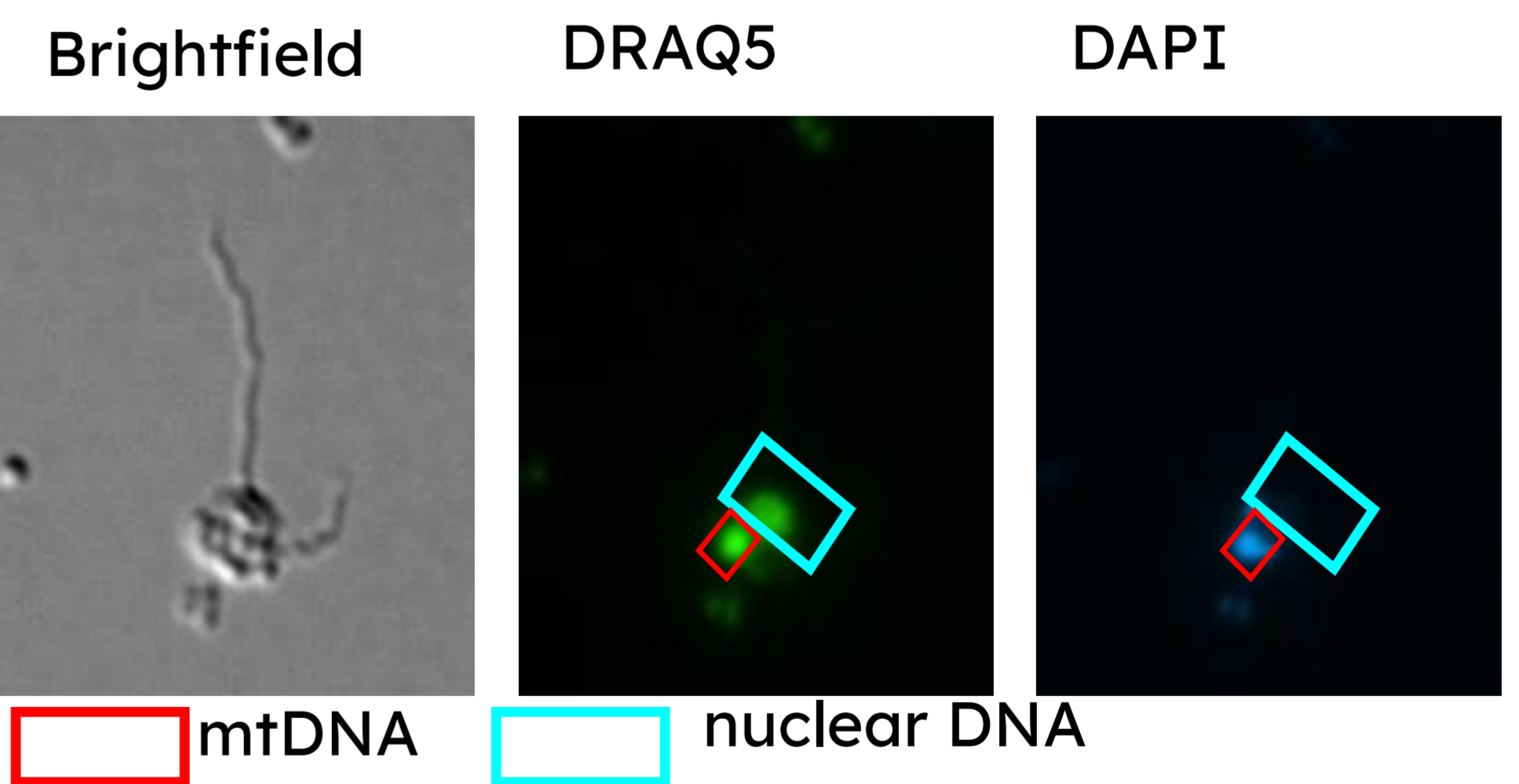


Automated quantification of DNA content in nuclei and mitochondria of Kinetoplastid protists

Vsevolod Kuksin (image analysis), Sofiia Porkhun, Sevinch Zaripova (image acquisition), Borys Olifirov (primary script for image analysis), Daryna Zavadzka (slide stainings)

Methods

DNA of kinetoplastids was stained by DAPI and DRAQ5. The preferential binding of DAPI with AT pairs and the fact that kDNA is usually AT-rich allowed to differentiate kDNA from nuclear DNA based on the fluorescence intensity of both structures in DAPI channel. Python script was used for automated quantification of nuclear and kDNA content.



Results & conclusions

The script is capable of automatically detecting cells based on the brightfield image; differentiating 3 different species of kinetoplastids (*Cruzella marina* and 2 distantly related isolates from *Neobodo designis* species complex) based on the estimated cell volume and DNA content; The preliminary estimates from a few draft microphotographs suggest that the amount of mt and nuclear DNA is lower in 2 representatives of *Neobodo designis* species complex if compared to *Cruzella marina*; moreover, the ratio of nuclear DNA content to mtDNA content consistently differs across species.

