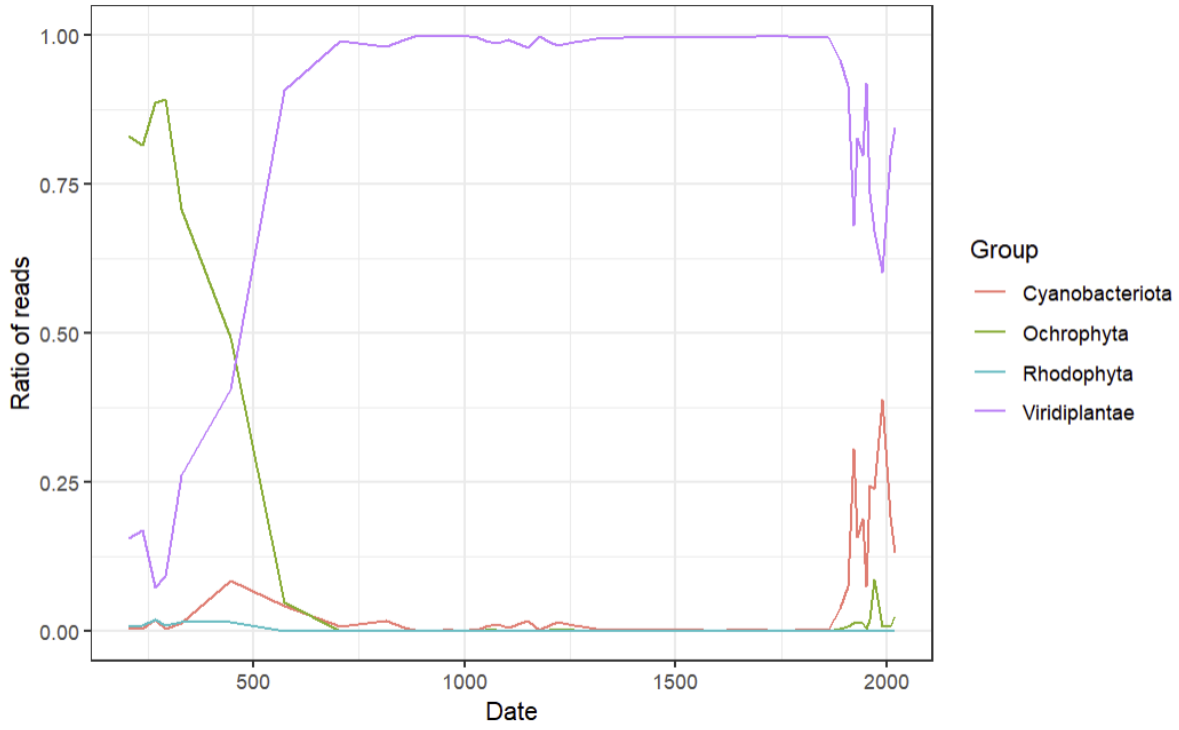
Method

The Tjörnin lake samples were also investigated for changes in species composition and chloroplast ratios over time. For each sample, the number of reads aligned to the photosynthetic taxa Viridiplantae, Cyanobacteria, Ochrophyta, and Rhodophyta was divided by the sum of reads aligned to the four groups, thereby returning the ratio of reads from each group (photo_org_C.sh). A Viridiplantae chloroplast ratio for each sample was directly calculated as the number of chloroplasts aligned reads divided by the number of Viridiplantae competitively aligned reads for each sample. The taxa ratios and chloroplast ratios were plotted over time using the ggplot2 R package (Plastid_ratios.Rmd).

Results

Tjörnin lake was originally a marine environment before 660 CE and became heavily polluted after 1750 CE due to urbanisation of the surrounding city of Reykjavík. These environmental changes had a large impact on the species composition of photosynthesisers in the lake[1]. This can be seen in the relative abundance of Viridiplantae aligned reads versus reads aligned to other photosynthetic taxa during competitive alignment (supplementary analysis fig 1).

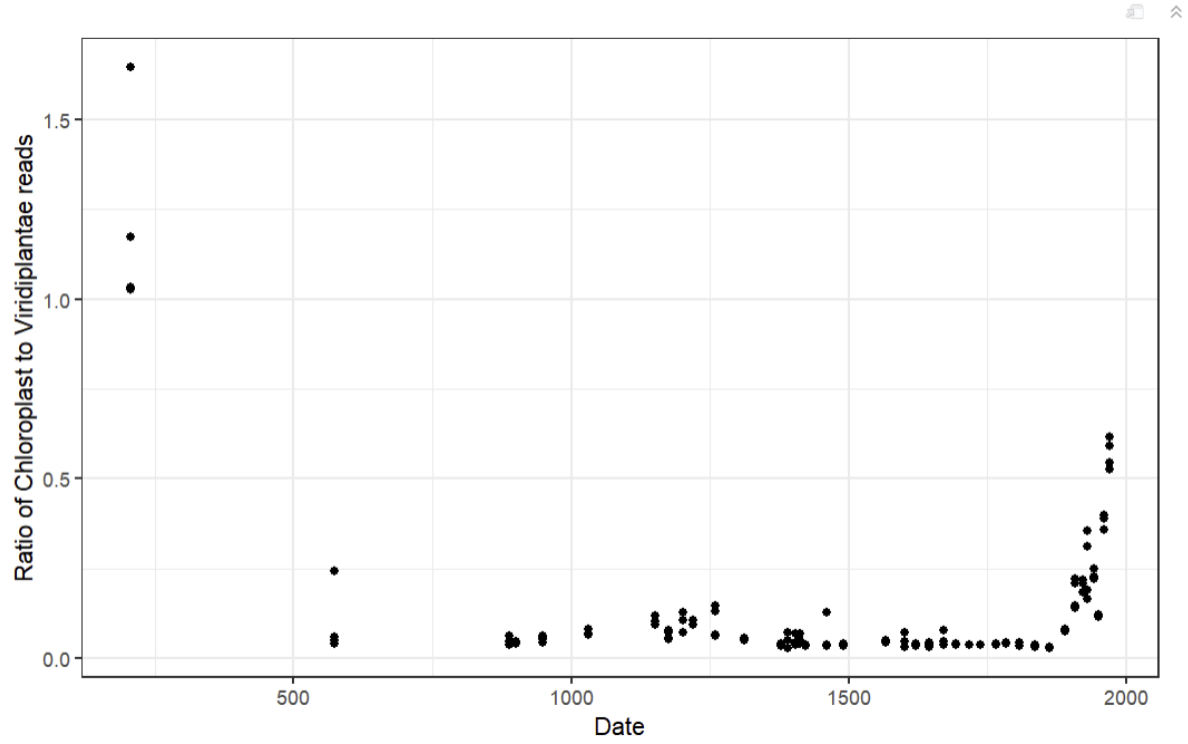


Supplement analysis figure 1: Graph of relative abundance of four photosynthetic clades in the Tjörnin lake samples. The x axis is the CE dating of the sample. The y axis is the proportion of reads aligned to each of the four groups, calculated as the number of reads assigned to the particular group divided by the summed number of reads assigned to any of the groups.

In the earliest measured dates, the lake samples are dominated by Ochrophyta reads, perhaps originating from marine algae. Between 660 CE 1890 CE and, the samples contain Viridiplantae reads almost exclusively, and in recent centuries, cyanobacteria begin to have a larger presence, likely due to increased pollution in the lake[1].

All chloroplasts are thought to originate from a single cyanobacterium that was engulfed by the ancestor of Viridiplantae and Rhodophyta in an endosymbiosis event[2]. The ancestor of ochrophytes later gained a chloroplast by endosymbiosis of a rhodophyte[3]. Cyanobacteria and chloroplasts from different algal taxa therefore share an evolutionary origin, as well as several conserved genes[2]. When the Tjörnin lake reads were aligned against Viridiplantae chloroplasts exclusively, it may be that reads from cyanobacteria and Ochrophyta chloroplasts aligned against the Viridiplantae chloroplasts for lack of a closer reference, whereas in the competitive mapping alignment, these reads could instead align to the correct taxa. During the periods where other groups of photosynthetic organisms were common, this could falsely inflate the

number of “Viridiplantae” chloroplast assigned reads compared to the competitively aligned reads, and is a possible explanation for how the ratio of Viridiplantae chloroplast reads to total Viridiplantae reads could exceed 1.00. The chloroplast ratio is indeed higher before 660 CE and after 1800 CE, when the ratio of Viridiplantae reads to other photosynthetic species was lower (supplementary analysis fig 2).



Supplementary analysis figure 2: Graph of chloroplast reads ratios and sample age in the Tjörnin lake samples. Each dot represents a sample. The x axis is the CE dating of the sample. The y axis is the ratio for Viridiplantae chloroplast reads to overall Viridiplantae reads in the sample.

In the future, a more accurate chloroplast ratio could possibly be obtained by extracting all Viridiplantae chloroplast aligned reads from competitive mapping, instead of separately aligning reads only to chloroplasts. This approach would require a list of all chloroplast accessions in the competitive mapping database, which was not available for this project.

References

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2. Ochoa De Alda JAG, Esteban R, Diago ML, Houmard J. The plastid ancestor originated among one of the major cyanobacterial lineages. *Nature Communications 2014 5:1*. 2014;5(1):4937-. doi:10.1038/ncomms5937

3. Strassert JFH, Irisarri I, Williams TA, Burki F. A molecular timescale for eukaryote evolution with implications for the origin of red algal-derived plastids. *Nature Communications 2021 12:1*. 2021;12(1):1879-. doi:10.1038/s41467-021-22044-z