Supplementary Text S1: Assessing the completeness of Chlorophyta genomes

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```
library(here)
library(cogeqc)
library(tidyverse)
library(Herper)

set.seed(123) # for reproducibility
options(timeout = 6000) # to load files from the web
```

1 Overview

Here, we will use *cogeqc* to assess the completeness of Chlorophyta genomes available on Pico-PLAZA 3.0 (Van Bel et al. 2018) using Best Universal Single-Copy Orthologs (BUSCO).

2 Managing external dependencies with virtual environments

Here, for convenience, we will install BUSCO in a Conda environment for use with *cogeqc*. For that, we will use the Bioconductor package *Herper*

Below, you can find the code to install miniconda in a directory of your choice (here, "~/Documents") and create a virtual environment containing a BUSCO installation.

```
# Path to where BUSCO will be installed and env name
my_miniconda <- file.path("~/Documents", "miniconda")
env <- "cogeqc_env"

# Create env named `cogeqc_env` with BUSCO in it
install_CondaTools(
    tools = "busco==5.3.0",
    env = env,
    channels = c("conda-forge", "bioconda"),
    pathToMiniConda = my_miniconda
)
```

3 Data acquisition

Now, we will load all genomes directly from PLAZA as `DNAStringSet` objects and export them to a single directory of FASTA files, so we can run BUSCO in batch mode.

```
# Links to Chlorophyta genomes from Pico-PLAZA 3.0
base_url <- "ftp://ftp.psb.ugent.be/pub/plaza/plaza_pico_03/Genomes/"
links <- paste0(
    base_url,
    c("mpu.fasta.gz", "mrcc299.fasta.gz", "olu.fasta.gz", "ome.fasta.gz",
      "orcc809.fasta.gz", "ota.fasta.gz", "bprrcc1105.fasta.gz",
      "cre.fasta.gz", "vca.fasta.gz", "cvu.fasta.gz", "acg.fasta.gz",
      "pse3.fasta.gz", "prcc4223.fasta.gz", "cnc64a.fasta.gz",
```

```
      "hsp.fasta.gz", "apr.fasta.gz")
)

# Load all genomes
genomes <- lapply(links, Biostrings::readDNAStringSet)
names(genomes) <- basename(links)

# Write all genomes to a subdirectory of tempdir
genomes_path <- file.path(tempdir(), "genomes")
if(!dir.exists(genomes_path)) { fs::dir_create(genomes_path) }

write <- lapply(seq_along(genomes), function(x) {
    Biostrings::writeXStringSet(
        x = genomes[[x]],
        filepath = file.path(genomes_path, names(genomes)[x])
    )
    return(NULL)
})

dir(genomes_path)
##  [1] "acg.fasta.gz"        "apr.fasta.gz"        "bprrcc1105.fasta.gz"
##  [4] "cnc64a.fasta.gz"     "cre.fasta.gz"        "cvu.fasta.gz"
##  [7] "hsp.fasta.gz"        "mpu.fasta.gz"        "mrcc299.fasta.gz"
## [10] "olu.fasta.gz"        "ome.fasta.gz"        "orcc809.fasta.gz"
## [13] "ota.fasta.gz"        "prcc4223.fasta.gz"   "pse3.fasta.gz"
## [16] "vca.fasta.gz"
```

4 Running BUSCO

Now that all genomes are stored as FASTA files in /tmp/Rtmp1XXP35/genomes, we can assess their completeness with BUSCO.

```
# See all possible lineage datasets
with_CondaEnv(
    env, list_busco_datasets(), my_miniconda
)

# Run BUSCO using chlorophyta_odb10 as the lineage data set
busco <- with_CondaEnv(
    env,
    run_busco(
        sequence = genomes_path,
        outlabel = "chlorophyta_busco",
        mode = "genome",
        lineage = "chlorophyta_odb10",
        outpath = tempdir(),
        download_path = tempdir()
    ),
    my_miniconda
)
```

```
# Read and parse the output
outdir <- file.path(tempdir(), "chlorophyta_busco")
busco_summary <- read_busco(outdir)
save(
    busco_summary,
    file = here::here("products", "result_files", "busco_summary.rda"),
    compress = "xz"
)
```

The parsed BUSCO output (as returned by `read_busco()`) looks like this:

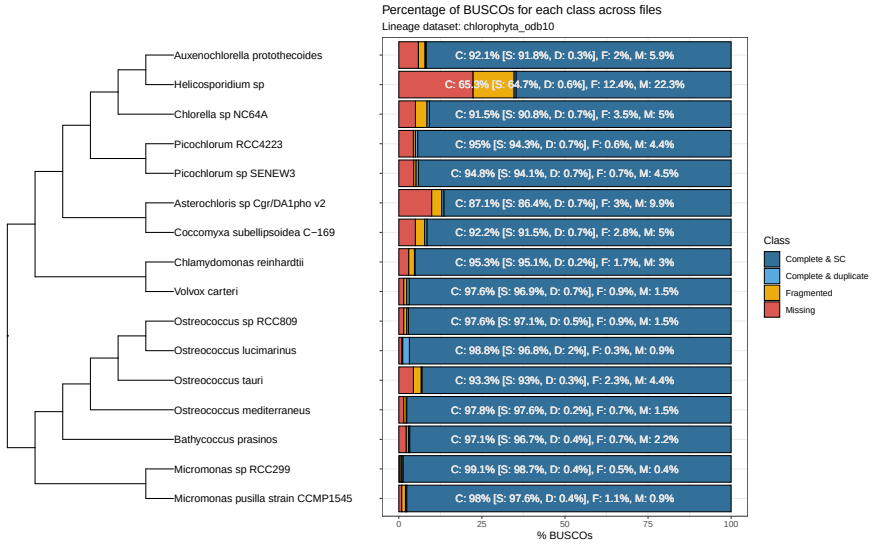
```
load(here("products", "result_files", "busco_summary.rda"))
head(busco_summary)
##         Class Frequency           Lineage             File
## 1 Complete_SC      94.1 chlorophyta_odb10    pse3.fasta.gz
## 2 Complete_SC      95.1 chlorophyta_odb10     cre.fasta.gz
## 3 Complete_SC      96.8 chlorophyta_odb10     olu.fasta.gz
## 4 Complete_SC      98.7 chlorophyta_odb10 mrcc299.fasta.gz
## 5 Complete_SC      91.8 chlorophyta_odb10     apr.fasta.gz
## 6 Complete_SC      86.4 chlorophyta_odb10     acg.fasta.gz
```

5 Visualizing summary statistics

Finally, let's visualize summary BUSCO stats:

```
# Manually create tree based on Pico-PLAZA's tree
c_branches <- function(b1, b2) {
    x <- paste0("(", b1, ",", b2, ")")
}

ostreococcus_root <- "((((Ostreococcus_lucimarinus, Ostreococcus_sp_RCC809), Ostreococcus_tauri), Ostreococcu
micromonas <- "(Micromonas_pusilla_strain_CCMP1545, Micromonas_sp_RCC299)"
chlamydomonadales <- "(Volvox_carteri, Chlamydomonas_reinhardtii)"
picochlorum <- "(Picochlorum_sp_SENEW3, Picochlorum_RCC4223)"
chlorellales <- "((Helicosporidium_sp, Auxenochlorella_protothecoides), Chlorella_sp_NC64A)"
trebouxiophyceae <- c_branches(
    "(Coccomyxa_subellipsoidea_C-169, Asterochloris_sp_Cgr/DA1pho_v2)",
    c_branches(picochlorum, chlorellales)
)

chlo_tree <- c_branches(
    c_branches(
        ostreococcus_root, micromonas
    ),
    c_branches(
        chlamydomonadales, trebouxiophyceae
    )
)
chlo_tree <- paste0(chlo_tree, ";")
```

```
# Read tree as a phylo object and clean species names
chlo_tree <- treeio::read.tree(text = chlo_tree)
chlo_tree$tip.label <- gsub("_", " ", chlo_tree$tip.label)

# Plot species tree and get species order from tree topology
p_tree <- plot_species_tree(chlo_tree, xlim = c(0, 12))
taxa_order <- rev(ggtree::get_taxa_name(p_tree))

# Plot BUSCO summary stats
p_busco <- busco_summary %>%
    mutate(File = str_replace_all(File, "\\.fasta.*", "")) %>%
    mutate(File = str_replace_all(
        File,
        c(
            "pse3" = "Picochlorum sp SENEW3",
            "cre" = "Chlamydomonas reinhardtii",
            "olu" = "Ostreococcus lucimarinus",
            "mrcc299" = "Micromonas sp RCC299",
            "apr" = "Auxenochlorella protothecoides",
            "acg" = "Asterochloris sp Cgr/DA1pho v2",
            "cvu" = "Coccomyxa subellipsoidea C-169",
            "bprrcc1105" = "Bathycoccus prasinos",
            "orcc809" = "Ostreococcus sp RCC809",
            "prcc4223" = "Picochlorum RCC4223",
            "ota" = "Ostreococcus tauri",
            "hsp" = "Helicosporidium sp",
            "mpu" = "Micromonas pusilla strain CCMP1545",
            "vca" = "Volvox carteri",
            "ome" = "Ostreococcus mediterraneus",
            "cnc64a" = "Chlorella sp NC64A"
        )
    )) %>%
    mutate(File = factor(File, taxa_order)) %>%
    plot_busco() +
    theme(axis.text.y = element_blank()) +
    labs(y = "")

# Combining phylogeny with BUSCO plot
combined <- patchwork::wrap_plots(p_tree, p_busco)
combined
```



Except for *Helicosporidium sp.*, Chlorophyta genomes on Pico-PLAZA 3.0 have a high quality, as denoted by their high completeness.

Session info

This document was created under the following conditions:

```
sessioninfo::session_info()
## - Session info -------------------------------------------------------------
##  setting  value
##  version  R version 4.2.1 (2022-06-23)
##  os       Ubuntu 20.04.4 LTS
##  system   x86_64, linux-gnu
##  ui       X11
##  language (EN)
##  collate  en_US.UTF-8
##  ctype    en_US.UTF-8
##  tz       Europe/Brussels
##  date     2022-10-04
##  pandoc   2.18 @ /usr/lib/rstudio/bin/quarto/bin/tools/ (via rmarkdown)
##
## - Packages -----------------------------------------------------------------
##  package          * version  date (UTC) lib source
##  ape                5.6-2    2022-03-02 [1] CRAN (R 4.2.0)
##  aplot              0.1.6    2022-06-03 [1] CRAN (R 4.2.0)
##  assertthat         0.2.1    2019-03-21 [1] CRAN (R 4.2.0)
##  backports          1.4.1    2021-12-13 [1] CRAN (R 4.2.0)
##  BiocGenerics       0.42.0   2022-04-26 [1] Bioconductor
##  BiocManager        1.30.18  2022-05-18 [1] CRAN (R 4.2.0)
##  BiocStyle        * 2.25.0   2022-06-15 [1] Github (Bioconductor/BiocStyle@7150c28)
##  Biostrings         2.64.1   2022-08-18 [1] Bioconductor
##  bitops             1.0-7    2021-04-24 [1] CRAN (R 4.2.0)
```

```
##  bookdown          0.27     2022-06-14 [1] CRAN (R 4.2.0)
##  broom             0.8.0    2022-04-13 [1] CRAN (R 4.2.0)
##  cellranger        1.1.0    2016-07-27 [1] CRAN (R 4.2.0)
##  cli               3.4.1    2022-09-23 [1] CRAN (R 4.2.1)
##  cogeqc          * 1.1.6    2022-10-04 [1] Github (almeidasilvaf/cogeqc@e86df13)
##  colorspace        2.0-3    2022-02-21 [1] CRAN (R 4.2.0)
##  crayon            1.5.2    2022-09-29 [1] CRAN (R 4.2.1)
##  DBI               1.1.3    2022-06-18 [1] CRAN (R 4.2.0)
##  dbplyr            2.2.1    2022-06-27 [1] CRAN (R 4.2.1)
##  digest            0.6.29   2021-12-01 [1] CRAN (R 4.2.0)
##  dplyr           * 1.0.10   2022-09-01 [1] CRAN (R 4.2.1)
##  ellipsis          0.3.2    2021-04-29 [1] CRAN (R 4.2.0)
##  evaluate          0.16     2022-08-09 [1] CRAN (R 4.2.1)
##  fansi             1.0.3    2022-03-24 [1] CRAN (R 4.2.0)
##  farver            2.1.0    2021-02-28 [1] CRAN (R 4.2.0)
##  fastmap           1.1.0    2021-01-25 [1] CRAN (R 4.2.0)
##  forcats         * 0.5.1    2021-01-27 [1] CRAN (R 4.2.0)
##  fs                1.5.2    2021-12-08 [1] CRAN (R 4.2.0)
##  generics          0.1.3    2022-07-05 [1] CRAN (R 4.2.1)
##  GenomeInfoDb      1.32.4   2022-09-06 [1] Bioconductor
##  GenomeInfoDbData  1.2.8    2022-05-06 [1] Bioconductor
##  ggfun             0.0.6    2022-04-01 [1] CRAN (R 4.2.0)
##  ggplot2         * 3.3.6    2022-05-03 [1] CRAN (R 4.2.0)
##  ggplotify         0.1.0    2021-09-02 [1] CRAN (R 4.2.0)
##  ggtree            3.4.0    2022-04-26 [1] Bioconductor
##  glue              1.6.2    2022-02-24 [1] CRAN (R 4.2.0)
##  gridGraphics      0.5-1    2020-12-13 [1] CRAN (R 4.2.0)
##  gtable            0.3.0    2019-03-25 [1] CRAN (R 4.2.0)
##  haven             2.5.0    2022-04-15 [1] CRAN (R 4.2.0)
##  here            * 1.0.1    2020-12-13 [1] CRAN (R 4.2.0)
##  Herper          * 1.1.2    2022-05-18 [1] Github (RockefellerUniversity/Herper@5bceeb4)
##  hms               1.1.1    2021-09-26 [1] CRAN (R 4.2.0)
##  htmltools         0.5.2    2021-08-25 [1] CRAN (R 4.2.0)
##  httr              1.4.4    2022-08-17 [1] CRAN (R 4.2.1)
##  igraph            1.3.2    2022-06-13 [1] CRAN (R 4.2.0)
##  IRanges           2.30.1   2022-08-18 [1] Bioconductor
##  jsonlite          1.8.2    2022-10-02 [1] CRAN (R 4.2.1)
##  knitr             1.40     2022-08-24 [1] CRAN (R 4.2.1)
##  labeling          0.4.2    2020-10-20 [1] CRAN (R 4.2.0)
##  lattice           0.20-45  2021-09-22 [1] CRAN (R 4.2.0)
##  lazyeval          0.2.2    2019-03-15 [1] CRAN (R 4.2.0)
##  lifecycle         1.0.2    2022-09-09 [1] CRAN (R 4.2.1)
##  lubridate         1.8.0    2021-10-07 [1] CRAN (R 4.2.0)
##  magrittr          2.0.3    2022-03-30 [1] CRAN (R 4.2.0)
##  Matrix            1.4-1    2022-03-23 [1] CRAN (R 4.2.0)
##  modelr            0.1.8    2020-05-19 [1] CRAN (R 4.2.0)
##  munsell           0.5.0    2018-06-12 [1] CRAN (R 4.2.0)
##  nlme              3.1-158  2022-06-15 [1] CRAN (R 4.2.0)
##  patchwork         1.1.1    2020-12-17 [1] CRAN (R 4.2.0)
##  pillar            1.8.1    2022-08-19 [1] CRAN (R 4.2.1)
##  pkgconfig         2.0.3    2019-09-22 [1] CRAN (R 4.2.0)
```

```
##  plyr              1.8.7    2022-03-24 [1] CRAN (R 4.2.0)
##  png               0.1-7    2013-12-03 [1] CRAN (R 4.2.0)
##  purrr           * 0.3.4    2020-04-17 [1] CRAN (R 4.2.0)
##  R6                2.5.1    2021-08-19 [1] CRAN (R 4.2.0)
##  Rcpp              1.0.9    2022-07-08 [1] CRAN (R 4.2.1)
##  RCurl             1.98-1.9 2022-10-03 [1] CRAN (R 4.2.1)
##  readr           * 2.1.2    2022-01-30 [1] CRAN (R 4.2.0)
##  readxl            1.4.0    2022-03-28 [1] CRAN (R 4.2.0)
##  reprex            2.0.1    2021-08-05 [1] CRAN (R 4.2.0)
##  reshape2          1.4.4    2020-04-09 [1] CRAN (R 4.2.0)
##  reticulate      * 1.25     2022-05-11 [1] CRAN (R 4.2.0)
##  rjson             0.2.21   2022-01-09 [1] CRAN (R 4.2.0)
##  rlang             1.0.6    2022-09-24 [1] CRAN (R 4.2.1)
##  rmarkdown         2.14     2022-04-25 [1] CRAN (R 4.2.0)
##  rprojroot         2.0.3    2022-04-02 [1] CRAN (R 4.2.0)
##  rstudioapi        0.13     2020-11-12 [1] CRAN (R 4.2.0)
##  rvest             1.0.2    2021-10-16 [1] CRAN (R 4.2.0)
##  S4Vectors         0.34.0   2022-04-26 [1] Bioconductor
##  scales            1.2.0    2022-04-13 [1] CRAN (R 4.2.0)
##  sessioninfo       1.2.2    2021-12-06 [1] CRAN (R 4.2.0)
##  stringi           1.7.8    2022-07-11 [1] CRAN (R 4.2.1)
##  stringr         * 1.4.1    2022-08-20 [1] CRAN (R 4.2.1)
##  tibble          * 3.1.8    2022-07-22 [1] CRAN (R 4.2.1)
##  tidyr           * 1.2.1    2022-09-08 [1] CRAN (R 4.2.1)
##  tidyselect        1.1.2    2022-02-21 [1] CRAN (R 4.2.0)
##  tidytree          0.3.9    2022-03-04 [1] CRAN (R 4.2.0)
##  tidyverse       * 1.3.1    2021-04-15 [1] CRAN (R 4.2.0)
##  treeio            1.20.0   2022-04-26 [1] Bioconductor
##  tzdb              0.3.0    2022-03-28 [1] CRAN (R 4.2.0)
##  utf8              1.2.2    2021-07-24 [1] CRAN (R 4.2.0)
##  vctrs             0.4.2    2022-09-29 [1] CRAN (R 4.2.1)
##  withr             2.5.0    2022-03-03 [1] CRAN (R 4.2.0)
##  xfun              0.33     2022-09-12 [1] CRAN (R 4.2.1)
##  xml2              1.3.3    2021-11-30 [1] CRAN (R 4.2.0)
##  XVector           0.36.0   2022-04-26 [1] Bioconductor
##  yaml              2.3.5    2022-02-21 [1] CRAN (R 4.2.0)
##  yulab.utils       0.0.4    2021-10-09 [1] CRAN (R 4.2.0)
##  zlibbioc          1.42.0   2022-04-26 [1] Bioconductor
##
##  [1] /home/faalm/R/x86_64-pc-linux-gnu-library/4.2
##  [2] /usr/local/lib/R/site-library
##  [3] /usr/lib/R/site-library
##  [4] /usr/lib/R/library
##
## ------------------------------------------------------------------------------
```

References

Van Bel, Michiel, Tim Diels, Emmelien Vancaester, Lukasz Kreft, Alexander Botzki, Yves Van de Peer, Frederik Coppens, and Klaas Vandepoele. 2018. "PLAZA 4.0: An Integrative Resource for Functional, Evolutionary and Comparative Plant Genomics." *Nucleic Acids Research* 46 (D1): D1190–96.