









Chlamydiales implication in the evolution of Archaeplastida

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Abstract Recently, a paradigm shift in the acquisition of photosynthesis has proposed the implication of an intracellular obligate pathogen in the primary plastid establishment (so far thought as an association between only two partners; a heterotrophic unicellular eukaryote and a cyanobacterium). This hypothesis, dubbed the Menage-a-trois Hypothesis (MATH), suggests an active and direct role of Chlamydiales in primary endosymbiosis, which would have provided many critical genes to the cyanobiont in the common inclusion vesicle. The expression and efficient localization of these genes, such as key transporters and glucan transferases, would have initiated the biochemical fluxes of symbiosis. Even if still controversial, the MATH is supported by molecular, biochemical and phylogenetic evidence. Hence, studies performed more than a decade ago concluded that 30-100 genes would have been transferred from Chlamydiales pathogens to the ancestor of Archaeplastida. In this work, we revisit the phylogenetic support for the MATH with the objective of updating the list of Chlamydiales genes found in modern Archaeplastida and identifying a congruent phylogenomic signal (if any) corresponding to the potential original pathogen. Starting from all relevant publicly available data, we produced a representative set of primary algae and Chlamydiales genomes, supplemented by non-photosynthetic eukaryotic and bacterial genomes. Selected proteomes were compared to each other and their proteins grouped into orthologous groups. Single-gene phylogenetic analyses then allowed us to automatically identify trees suggesting a Chlamydiales origin of the Archaeplastida proteins. By this way, we were able to identify about 150 genes (of which 40-50 identified in the original studies) that may have been transferred from Chlamydiales during plastid establishment. Manual analyses are nevertheless necessary to confirm this number. A second round of phylogenomic analyses is ongoing, either based on explicit subsets of concatenated congruent gene alignments or using a Bayesian model that automatically clusters genes based on shared histories.

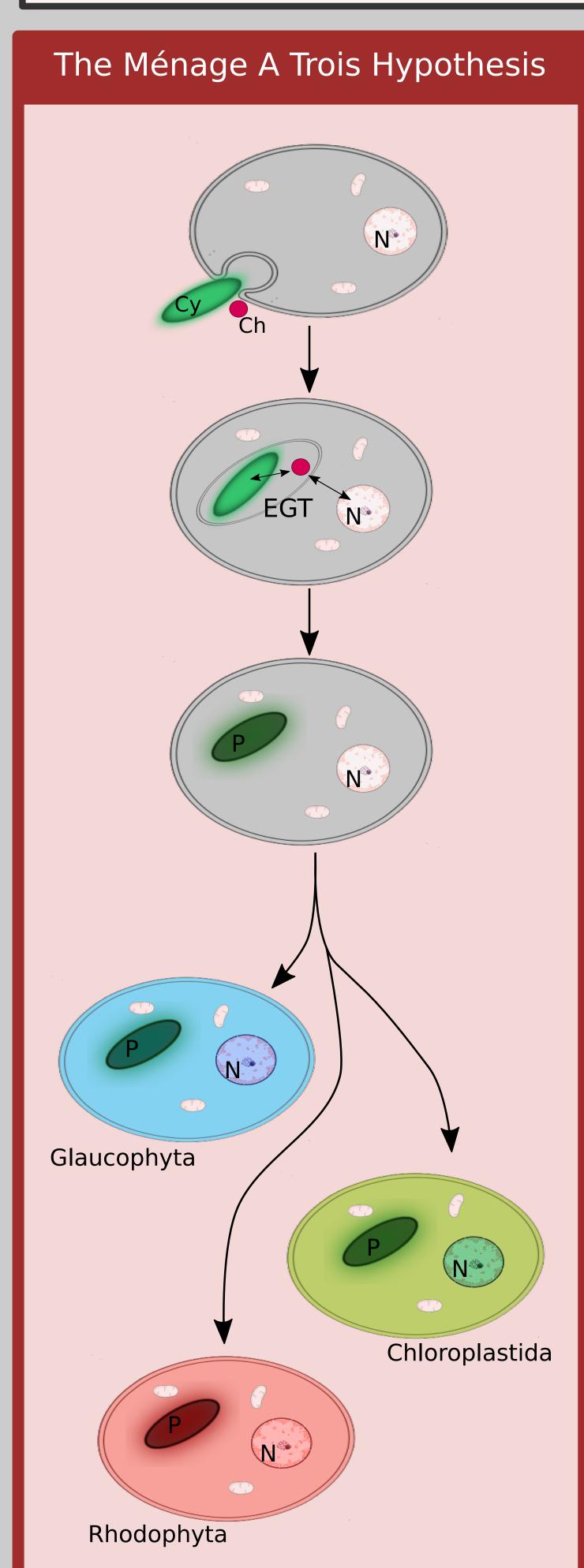


Figure 1: Schematic representation of The Ménage A Trois Hypothesis. This hypothesis suggests the involvement of a pathogen related to Chlamydia (Ch) in the primary endosymbiosis of the ancestral cyanobacterium (Cy) by the eukaryote, during the first steps of plastid establishment. Several genes would have been transfered from the Chlamydia to the nucleus of the eukaryotic cell (N) and to the future plastid (P) (black arrows). Primary endosymbiosis of the plastid is at the origin of the three lineages of Archaeplastida: Glaucophyta, Rhodophyta and Chloroplastida. EGT: Endosymbiotic Gene Transfer

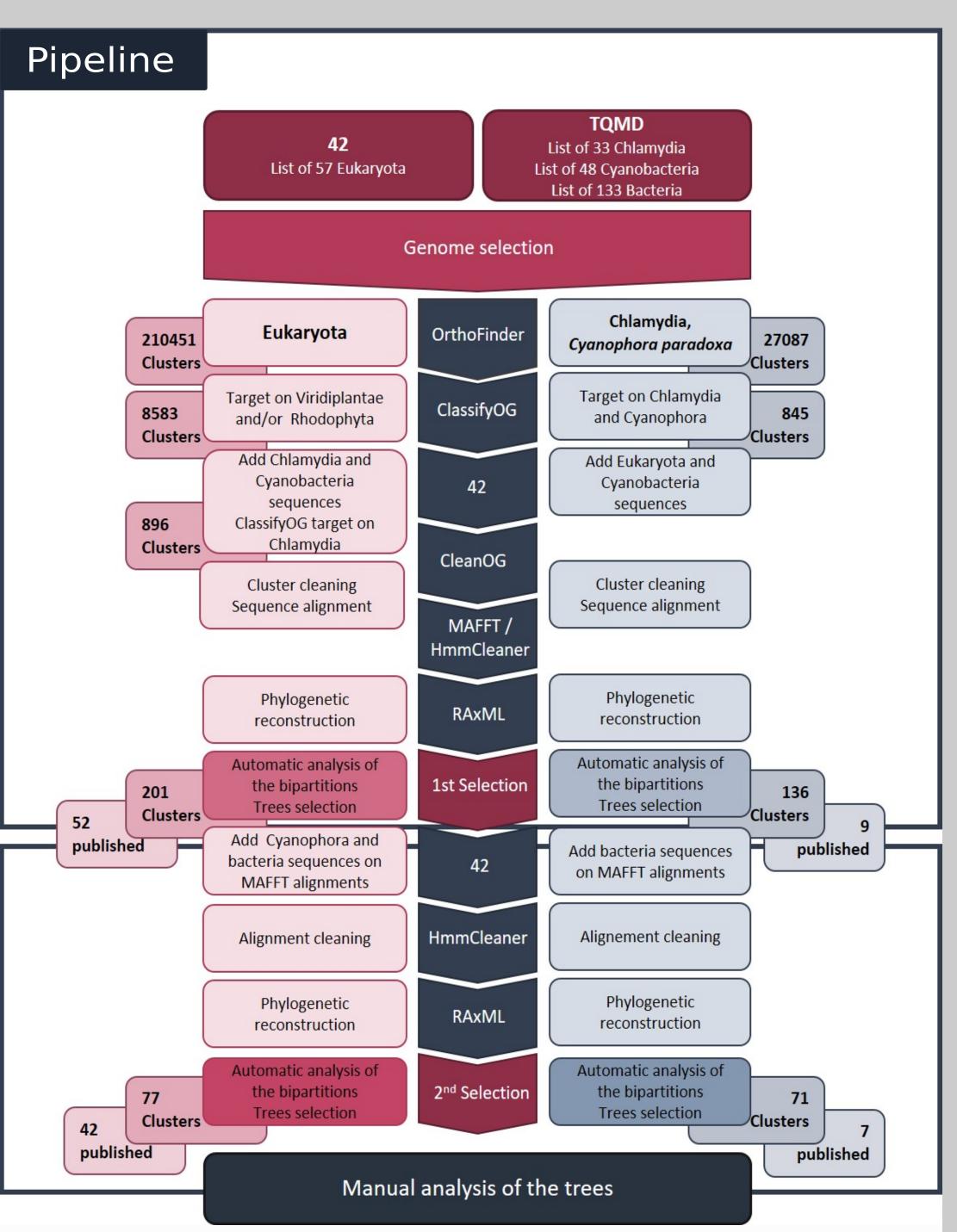
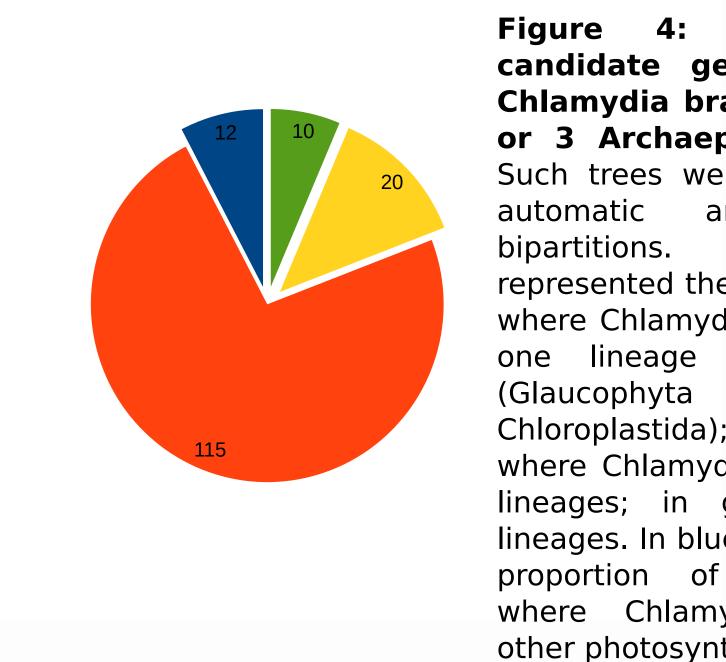


Figure 2: Flowchart of the bioinformatic pipeline used for the identification of putative chlamydial gene transfer candidates in Archaeplastida.



candidate gene trees where Chlamydia branch with 0, 1, 2, or 3 Archaeplastida lineages. Such trees were selected by the analysis In orange represented the proportion of trees where Chlamydia branch with only one lineage of Archaeplastida Rhodophyta Chloroplastida); in yellow, the trees where Chlamydia branch with two lineages; in green, with three lineages. In blue is represented the proportion of candidate trees where Chlamydia branch with other photosynthetic organisms.

Proportions

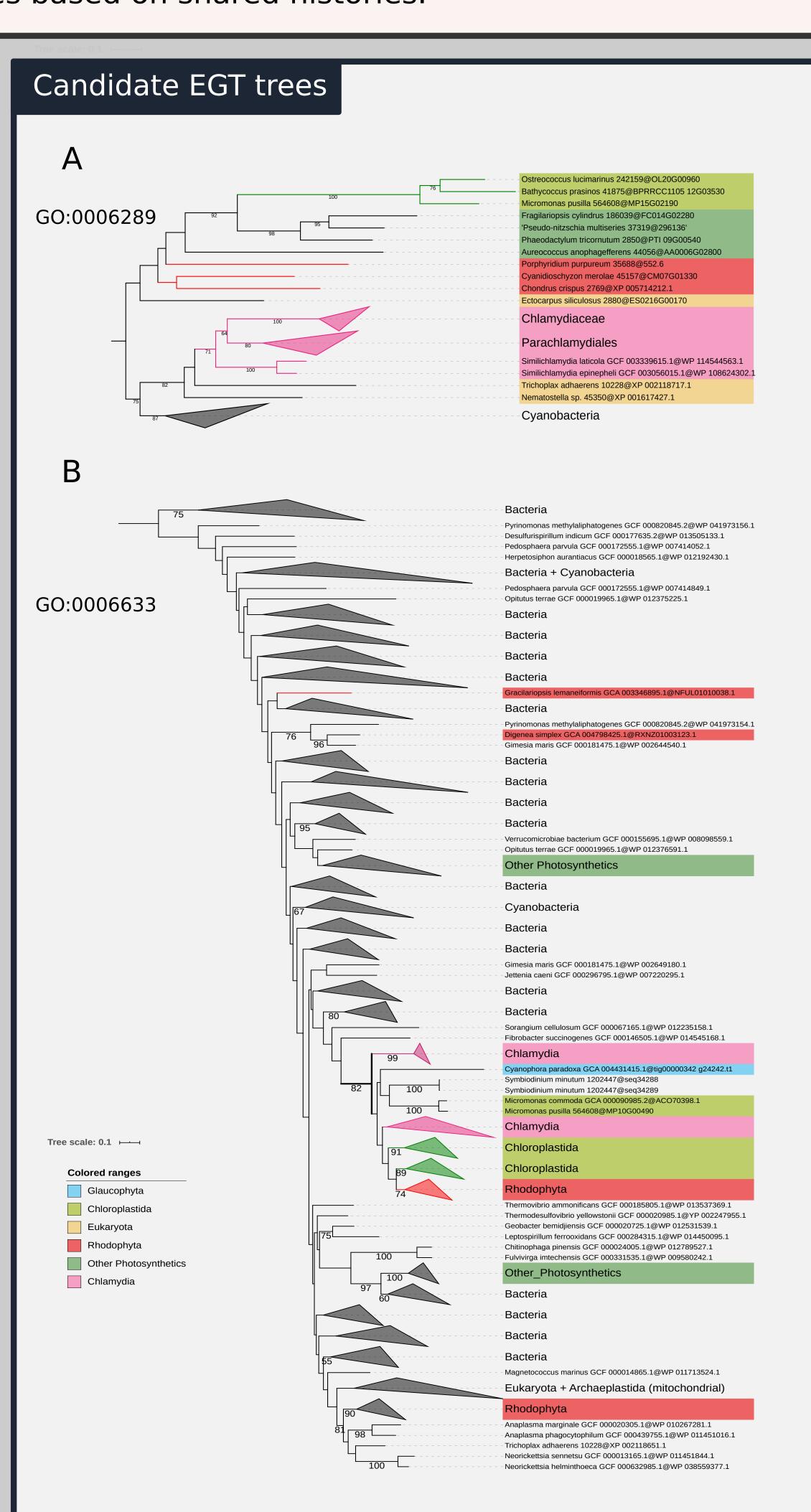


Figure 3: Single gene phylogenetic trees obtained through our selection pipeline. The pipeline discriminates two types of trees: the ones in which Chlamydia and Archaeplastida do not branched together (A), and the ones in which at least one Chlamydia is phylogenetically associated with at least one Archaeplastida (B). Bootstraps (>50) are indicated. The color key for both trees is given on the left.

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Conclusion

This project aims to test The Ménage à Trois hypothesis, more specifically to determine the implication of Chlamydia in plastid establishment. Thus, the first step was to identify all putative gene transfers between Chlamydia and Archaeplastida. This bioinformatics study, using all public data and multi-step phylogenetic pipeline, allowed us to identify about 150 genes, of which about 50 had already been identified in previous studies, showing a phylogenetic association between Chlamydia and Archaeplastida. Supplementary analyses are ongoing to confirm this number and determine if those genes may have been transferred during primary plastid endosymbiosis.