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MESOSTIGMA VIRIDE, OSTREOCOCCUS TAURI, AND THE ORIGIN OF GREEN PLANTS – A PHYLOGENETIC ASSESSMENT

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Abstract: Green plants (*Virideplantae*) are represented by two main lineages: the *Streptophyta* and the *Chlorophyta*. Chlorophytes comprise the majority of green algae while streptophytes include the charophyte algae and the embryophytes. Ultrastructural studies as well as phylogenetic analyses of several nuclear encoded genes suggested that the unicellular alga *Mesostigma viride* represent the closest relative of streptophytes. However, other studies, employing plastid and mitochondrial genes, suggested that *Mesostigma* represents a lineage that emerged before the divergence of the *Streptophyta* and *Chlorophyta.* Recently, the genome of the prasinophyte *Ostreococcus tauri,* which holds a basal position in the evolution of *Chlorophyta,* has been sequenced. To gain more insight on the phylogenetic positioning of the unicellular algae *M. viride* and *O. tauri* on the tree of green plants we analyzed phylogenetically three proteins of rather different origin, function and intracellular localization. Analyses were performed using two methods of phylogenetic reconstruction: Neighbor-Joining and Bayesian inference. The analysis of the ω-6 fatty acid desaturase, an enzyme of cyanobacterial origin, places *M. viride* at the base of the streptophytes; homologous sequences seem to be absent in the *O. tauri* database. The phylogenetic reconstruction based on the subunit c of vacuolar ATPase, a protein restricted to eukaryotes, suggests that *O. tauri* might represent the ancestor of all green plants while *M. viride* that of the streptophytes. The analysis of the enzyme peroxireductase II, points toward a proteobacterial origin of this protein and places both, *O. tauri* and *M. viride*, at the base of all green plants. These results suggest that the positioning of *O. tauri* and *M. viride* on the tree of green plants is strongly influenced by the protein selected for analyses and by the absence/presence of sequences from other basal taxa; therefore, the hypothesis that *O. tauri* and *M. viride* might represent lineages that diverged before the occurrence of the two main lineages of green plants cannot be completely rejected. Also, our study underlines the fact that more sequences, from other basal groups of prasinophytes, such as the *Pyramidomonales* and *Prasinococcales*, are needed in order to unambiguously resolve the position of *M. viride* and *O. tauri* on the tree of green plants.

Keywords: *Mesostigma viride, Ostreococcus tauri, green algae, prasinophytes, origin of green plants, phylogeny.*

Introduction

The origin and later diversification of green plants (*Virideplantae*) represents one of the most important biological radiations. The two lineages of the green plants, the *Streptophyta* and *Chlorophyta* diverged around 1 billion years ago[1, 14, 30]. Chlorophytes comprise the majority of green algae while streptophytes include the charophyte algae and the embryophytes [18]. *Mesostigma viride* is considered one of the most primitive green alga; however, its phylogenetic placement is still subject of debate (for a review see 18). Ultrastructural characteristics [20] and phylogenetic analyses of several nuclear encoded genes [2, 15, 19, 23] have found *Mesostigma* as the closest relative of the streptophytes while studies of chloroplast and mitochondrial genes suggested that *Mesostigma* represents a lineage that emerged before the divergence of the *Streptophyta* and *Chlorophyta* [17, 37]. Recently, the genome of *Ostreococcus tauri*, a member of the polyphyletic group of prasinophytes, has been sequenced [6]. *Ostreococcus tauri* is the smallest described autotrophic eukaryote [4] and appears very close to *Mantoniella* (*Mamiellales*, *Prasinophyceae*, *Chlorophyta*), a typical scaly prasinophyte [3, 6, 11]. The position of the prasinophytes on the phylogenetic tree of green plants is still not clear; most studies suggest that this group diverged early in the evolution of *Chlorophyta* while other suggest that prasinophytes represent a sister group of *Streptophyta* and *Chlorophyta* (for a review see 18). So far, because of the scarcity of sequences available for *M. viride* and *O. tauri*, no thorough analyses, with the inclusion of data from both taxa, have been performed. However, the recent expressed sequence tags (ESTs) data produced for *M. viride* [32] and the sequencing of the *O. tauri* genome [6] will likely overcome this drawback. To gather more data on the phylogenetic and taxonomic positioning of the unicellular algae *M. viride* and *O. tauri* on the tree of green plants we decided to analyze phylogenetically three proteins of different origin, function and intracellular localization. Neighbor-Joining and Bayesian analyses of the ω-6 fatty acid desaturase, the subunit c of vacuolar ATPase (vATPase) and of the peroxiredoxin II places *M. viride* either at the base of the streptophyte clade or of all green plants while *O. tauri* was always basal to *M. viride.* These results do not refute the hypothesis that *O. tauri* and *M. viride* might represent lineages that diverged before the occurrence of the two main lineages of green plants and suggest that more sequences, from other basal groups of prasinophytes, are needed to unequivocally resolve the position of these two taxa on the tree of green plants.

Materials and Methods

Chlamydomonas reinhardtii sequences of ω-6 fatty acid desaturase, subunit c of the vATPase and thioredoxin peroxidase have been found in NCBI database or *Chlamydomonas* database - ChlamyDB (http://genome.jgi-psf.org/Chlre3/Chlre3.home.html). *Chlamydomonas reinhardtii* deduced amino acid sequences were then used as query sequences for BLAST searches in: (i) the NCBI database (BLASTP) to retrieve prokaryotic and eukaryotic homologous sequences; (ii) *Ostreococcus tauri* database (http://bioinformatics.psb.ugent.be/genomes.php, BLASTP) (iii) the NCBI database (TBLASTN; EST_others) to retrieve the homologous sequences (ESTs) from *Mesostigma viride* strain NIES 476. (iv) Taxonomicaly Broad EST database - TBestDB (http://amoebidia.bcm.umontreal.ca/pepdb/searches/login.php) to retrieve homologous sequences from *Acetabularia acetabulum, Bigelowiella natans, Chlamydomonas incerta* and *Scenedesmus obliquus*; the TBLASTN search against TBestDBpub was performed using Anabench (http://malawimonas.bcm.umontreal.ca/anabench). The ESTs from *Mesostigma viride* were clustered with DAMBE 4.2.13 [38] or BioEdit 7.0 [13] and the deduced amino acid sequence was used for phylogenetic analyses. The accession numbers of the sequences used in phylogenetic analyses are listed in Table 1. Multiple alignments were done using Clustal X [36] and edited with BioEdit 7.0 . Neighbor-Joining (NJ) and Bayesian analysis were performed with MEGA 3.1 [16] and Mr. Bayes 3.1 [28], respectively. The phylogenetic trees were displayed using TreeView [25]. The presence of the N-terminal extension, which is specific for proteins imported in the plastid, was assessed by multiple alignments, and TargetP, ChloroP, and Predotar [9, 10, 34] were used to determine if these extensions represent targeting peptides.

Results

Identification and phylogeny of the ω-6 fatty acid desaturase from *M. viride*. BLAST search in NCBI database identified 11 ESTs that encode for *M. viride* ω-6 fatty acid desaturase (Table 1**)**. Multiple alignments of the deduced amino acid resulted from clustering the 11 ESTs sequences indicated that we succeeded to retrieve the full sequence of *M. viride* ω-6 fatty acid desaturase; the protein has 452 amino acids, a molecular weight of 50950 Da and a theoretical pI of 8.78. The alignment of *M. viride* protein with cyanobacterial homologs revealed the presence of an N-terminal extension; TargetP, ChloroP, and Predotar [9, 10, 34] indicated this extension as a plastid-targeting sequence. The search of *O. tauri* database with *M. viride, C. reinhardtii* and cyanobacterial ω-6 fatty acid desaturases failed to identify any homologous sequence. The closest BLAST hits were for the entries Ot02g07730 and Ot17g02110, which have high similarity with a Δ12 fatty acid desaturase from the bacillariophyte *Phaeodactylum tricornutum*

(NCBI accession number AAO23565). Both methods of phylogenetic analysis succeeded to separate, with strong support, the two main lineages of green plants and place *M. viride* at the base of the streptophytes (Fig. 1).

Table 1: Acession numbers of ω-6 fatty acid desaturase, thioredoxin peroxidase and vATPase sequences used for phylogenetic analyses

GenBank accession numbers
ω-6 fatty acid desaturase: Arabidopsis thaliana (NP_194824); Brassica napus (P48627); Chlamydomonas reinhardtii (BAA23881 and BAA83822); Glycine max (P48628); Mesostigma viride strain NIES 476 (DN255204, DN263990, DN256288, DN263404, DN256786, DN257608, DN258514, DN263403, DN256003, DN257762 and DN263989); Nostoc punctiforme (ZP_00108583); Olea europaea (AAV41001); Oryza sativa (XP_482619); Spinacia oleracea (CAA55121); Synechocystis sp. (NP_441489). **vATPase, subunit c**: Arabidopsis thaliana (NM_106215); Aspergillus nidulans (XP_660692); Avena sativa (P23957); Avicennia marina (AAK01292); Candida tropicalis (Q00607); Heterocapsa triquetra (AAW79383); Mesostigma viride strain NIES 476 (DN257987, DN254417, DN255147, DN254439, DN256379, DN254762, DN256807, DN259263, DN259262 and DN255906); Nicotiana tabacum (CAA65062); Oryza sativa (AAU44174); Paramecium tetraurelia (CAH39842); Plantago major (CAH58637); Porphyra tenera (JC7151); Schizosaccharomyces pombe (CAB11240); Solanum tuberosum (ABB29937). **Peroxireductase II**: Agrobacterium tumefaciens (AAL41795); Arabidopsis thaliana (NP_190864 and AAM65848); Bartonella bacilliformis (ZP_00948150); Bordetella pertussis (CAE42992); Brucella melitensis (AAL52637); Capsicum annuum (AAL35363); Caulobacter crescentus (AAK25356); Dunaliella salina (BM448554); Lycopersicon esculentum (AAP34571); Mesostigma viride strain NIES 476 (DN257791, DN254272 and DN256575), Oryza sativa (NP_916886, BAD37738 and XP_464429); Paracoccus denitrificans (ZP_00631017); Rhodobacterales bacterium (ZP_01014823); Scherffelia dubia (AL132698); Synechocystis sp. (NP_441096); Trichodesmium erythraeum (ZP_00675690); Vibrio sp. (ZP_00761618).
Sequences from other databases
ω-6 fatty acid desaturase: Scenedesmus obiquus (451)[3]. **vATPase, subunit c**: Bigelowiella natans (258)[3], Chlamydomonas reinhardtii (159570)[2]; Chlamydomonas incerta (1359)[3]; Ostreococcus tauri (Ot07g02040)[2]; Scenedesmus obliquus (1200)[3]. **Peroxireductase II**: Acetabularia acetabulum (1271)[3]; Chlamydomonas reinhardtii(157174)[1]; Ostreococcus tauri (Ot12g00200)[2]; Scenedesmus obliquus (42)[3].

[1] protein ID; *Chlamydomonas reinhardtii* database (http://genome.jgi-psf.org/chlre2/chlre2.home.html)

[2] protein ID; *Ostreococcus tauri* database (http://bioinformatics.psb.ugent.be/genomes.php)

[3] cluster number; TBest database (http://amoebidia.bcm.umontreal.ca/pepdb/searches/login.php)

Identification and phylogeny of the subunit c of vacuolar ATPase from *M. viride* and *O. tauri*. The NCBI database search identified 3 *M. viride* ESTs that encode the subunit c of the V_o domain of vATPase (Table 1). Even though the number of ESTs identified was rather low it allowed the recovery of the full length sequence. In *M. viride* the subunit c has 163 amino acids, a molecular weight of 16393 Da and a theoretical pI of 7.77. BLASTP search of the *O. tauri* database identified a truncated polypeptide (entry Ot07g02040). Multiple alignments with other eukaryotic sequences revealed that the Methionine from the N-terminus is missing as well as the first 15-20 amino acids. Furthermore, the first 6 amino acids from *Ostreococcus* are different from the conserved consensus sequence suggesting that the sequence from *O. tauri* has a frame shift at its N-terminus; these 6 amino acids were not used in phylogenetic analyses. Both phylogenetic reconstructions, resulted from NJ and Bayesian analyses, respectively, place *Ostreococcus*, with good statistical support, at the base of the green plant clade and *M. viride* at base of the streptophyte clade (Fig. 2). The topology of the land plant clade was not well resolved by the NJ method (most of the bootstrap values were under 50) probably due to the low number of differences among the sequences belonging to these taxa.

Identification and phylogeny of the peroxieductase II from *M. viride* and *O. tauri*. The 10 ESTs corresponding to *M. viride* peroxireductase II identified in NCBI database (Table 1) allowed retrieving most of the coding sequence; multiple alignments with other algal and land plant sequences indicated that 10-15 AA are missing from the C-terminus. BLASTP search of the *O. tauri* database found the entry Ot12g00200 as the best hit; as a result, this sequence was used in phylogenetic analyses. The protein from *O. tauri* has 177 amino acids, a molecular weight of 19271 Da and a theoretical pI of 6.75. Multiple alignments showed that *A. acetabulum*, *C. reinhardtii*, *D. salina* and *S. obliquus* have extensions of 15-30 amino acids versus bacterial and cyanobacterial sequences while *M. viride*, *S. dubia* and several sequences from land plants (indicated in figure 3 with the letter P after the species name) have extension of 40-60 amino acids; no extension could be determined in *O. tauri* peroxireductase II. All the prediction programs used in this study, i.e., TargetP, ChloroP, and Predotar [9, 10, 34] indicated that the N-terminal extensions present in *M. viride* and land plants (peroxireductase IIE from *A. thaliana* and *O. oryza*) correspond to plastid-targeting sequences. The phylogenetic reconstruction was less successful for this enzyme. The NJ method failed to robustly solve the *Chlorophyta* clade and the position of *Mesostigma*. In contrast, Bayesian analyses provided a more reliable statistical support (Figure 3). *O. tauri* holds a position basal to all green plants but, surprisingly, also to a cluster comprising alpha-proteobacteria. *M. viride* and the prasinophyte *S. dubia* branches below the clades of the streptophytes and chlorophytes. Several alignments, including more or less sequences, as well as different trimmings of these sequences did not change significantly the topology of the tree presented in Figure 3. When sequences of mitochondrial peroxiredoxin from *A. thaliana* and *O. oryza* (peroxiredoxin IIF) were introduced in the Bayesian analysis the resulting tree showed that this sequences cluster with the alpha-proteobacteria *P. denitrificans*, *B. melitensis* and *A. tumefaciens* (data not shown).

Discussions

Recent papers leaned the balance towards a wide acceptance of the fact that *Mesostigma viride* is the ancestor of *Streptophyta* and not a lineage that emerged before the divergence of the *Streptophyta* and *Chlorophyta* [23, 26, 32]. However, with the exception of one paper [23] in which the search for homologous sequences *O. tauri* database was performed exhaustively, the other analyses did not consider including data from *O. tauri* or other prasinophytes, which represent the most basal group of chlorophytes.

To this end we decided to analyze phylogenetically three proteins of different origin, function and intracellular localization. In plants, fatty acid biosynthesis and several steps leading to the formation of various unsaturated fatty acids take place in plastids [22, 24]; ω-6 fatty acid desaturase, which catalyses the introduction of an omega-6 double bond into the fatty acid hydrocarbon chain is one of the enzymes involved in these processes [31, 35]. The limited phylogenetic analysis performed by [31], in which they included the sequence of *C. reinhardtii*, the only sequence from green algae available at that time, could only reveal that the desaturase from *C. reinhardtii* has affinities with both cyanobacterial and land plants sequences. In this study, other sequences from streptophytes and chlorophytes, including the full sequence from *M. viride*, were used, and the analysis revealed *M. viride* as the ancestor of the streptophytes. Noteworthy, BLAST search in *O. tauri* database failed to find any sequence related to green plant or cyanobacterial ω-6 fatty acid desaturase; this result suggests that either this sequence is not present in the minimal genome of *O. tauri* [3] or still awaits to be sequenced. The absence of homologs of *A. thaliana* or/and *C. reinhardtii* genes from *O. tauri* genome is not very surprising as the *O. tauri* genome is almost nine times smaller than that of *A. thaliana* (which has a minimal genome size for land plants) or *C. reinhardtii* [3, 12].

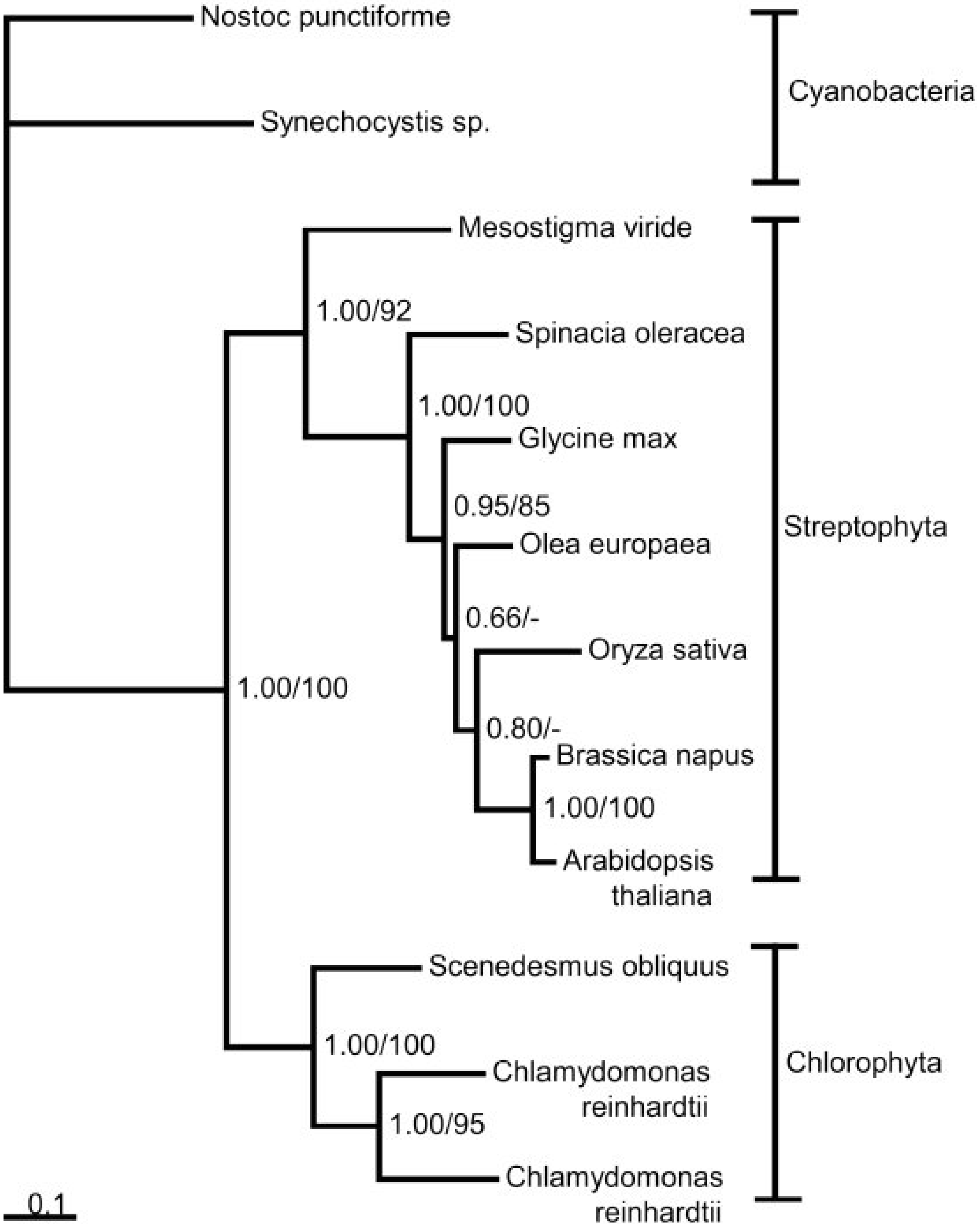

Fig. 1: Phylogenetic Bayesian consensus tree of the enzyme ω-6-fatty acid desaturase (mixed amino acid model; 500,000 generations; sample frequency=100; burnin=1,000) based on 12 sequences and 345 amino acid positions. The statistical support for internal nodes was determined by posterior probabilities (Bayesian inference) and bootstrap analyses (Neighbor-Joining) and is shown, in this order, at the corresponding branches. NJ support values ≤50% are indicated by (-). The tree resulted by Neighbor-Joining analysis (JTT+Γ4 model, pairwise deletion, bootstrap value=100) gave similar topology.

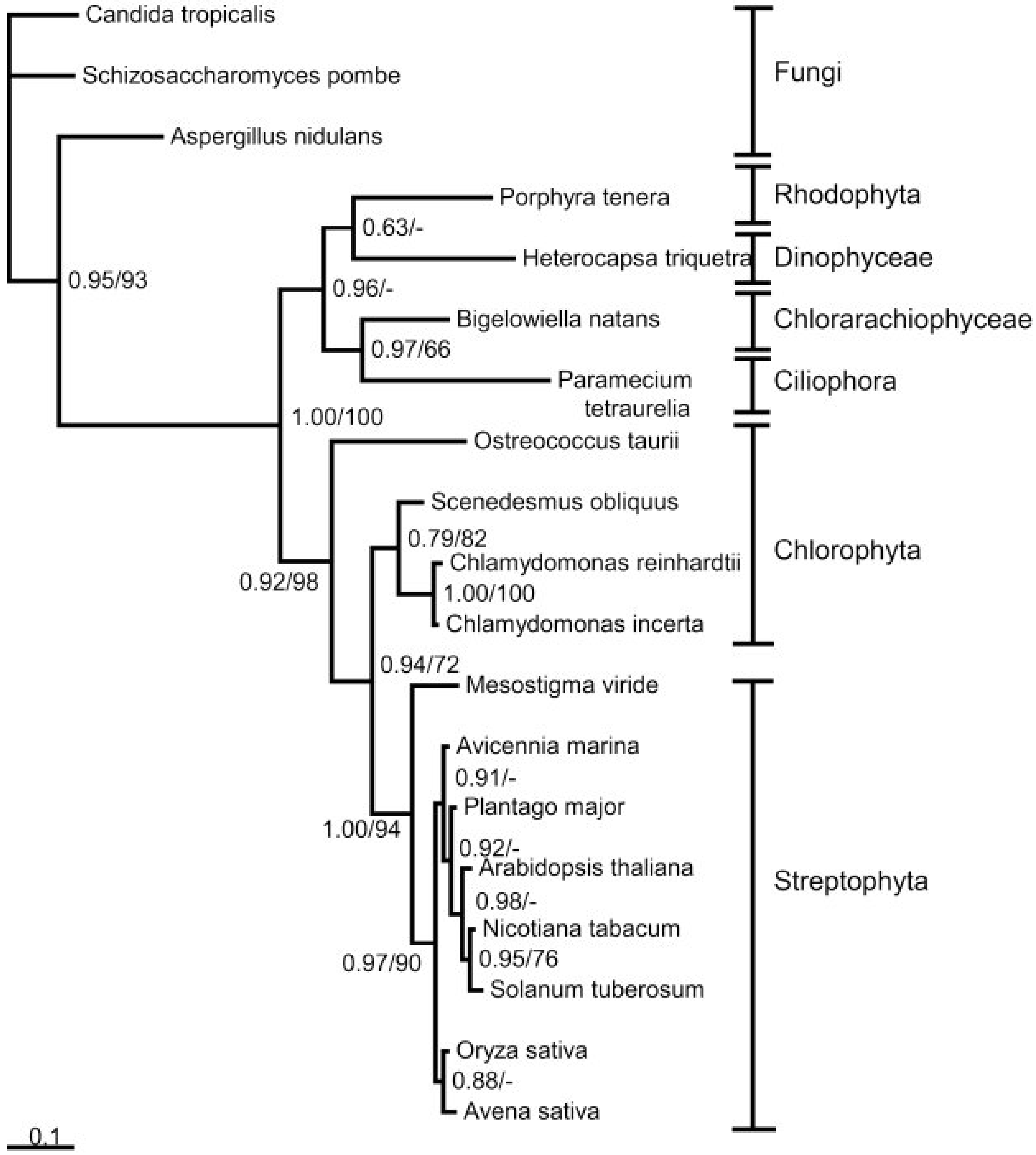

Fig. 2: Phylogenetic Bayesian consensus tree of subunit c of the vacuolar ATPase (mixed amino acid model; 500,000 generations; sample frequency=100; burnin=1,000) based on 19 sequences and 163 amino acid positions. The statistical support for internal nodes was determined by posterior probabilities (Bayesian inference) and bootstrap analyses (Neighbor-Joining) and is shown, in this order, at the corresponding branches. NJ support values ≤ 50% are indicated by (-). The tree resulted by Neighbor-Joining analysis (JTT+Γ4 model, pairwise deletion, bootstrap value=100) gave similar topology.

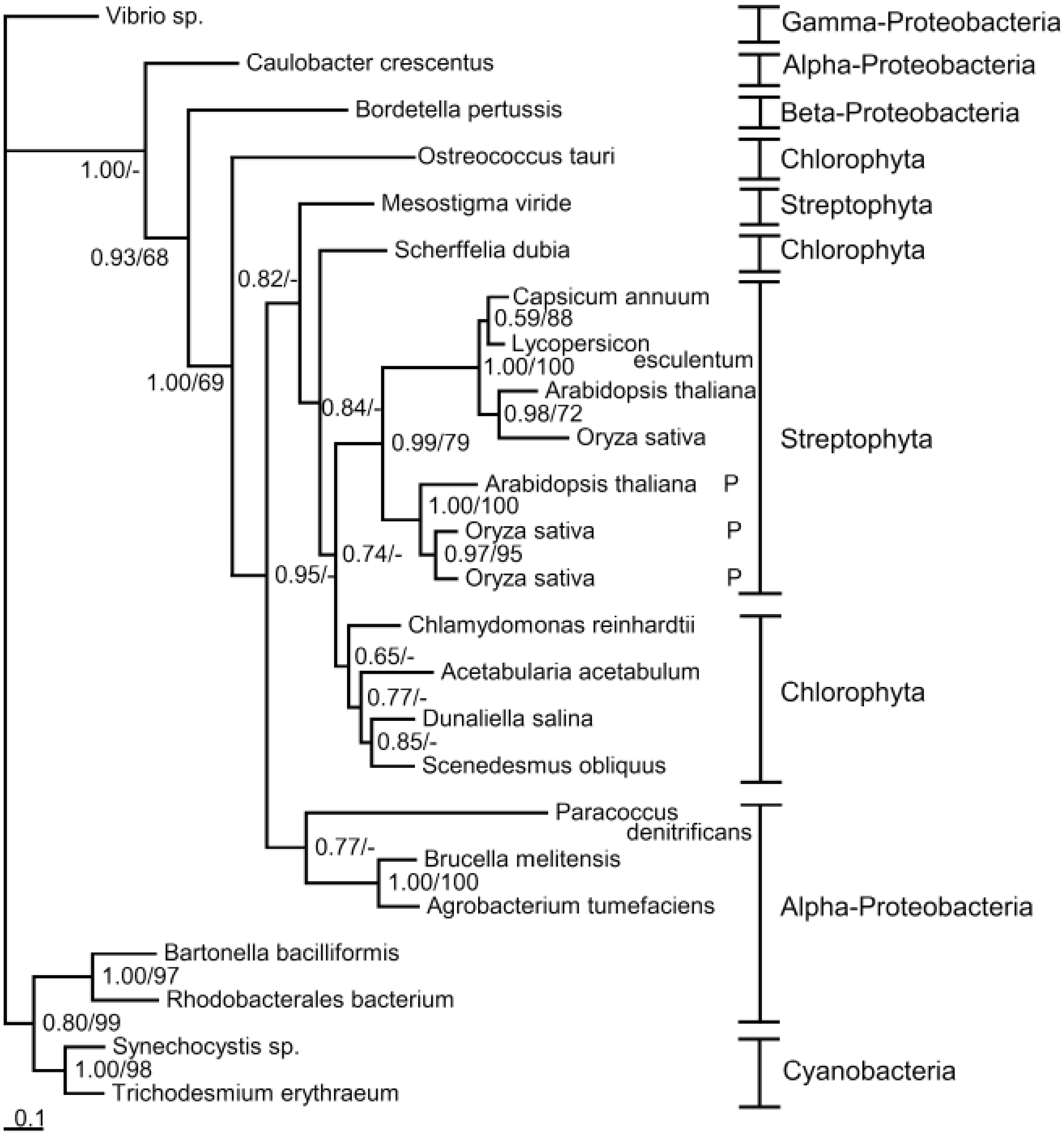

Fig. 3: Phylogenetic Bayesian consensus tree of the enzyme peroxiredoxin II (WAG+Γ4 model; 250,000 generations; sample frequency=100; burnin=500) based on 24 sequences and 161 amino acid positions. The statistical support for internal nodes was determined by posterior probabilities (Bayesian inference) and bootstrap analyses (Neighbor-Joining) and is shown, in this order, at the corresponding branches. NJ support values≤ 50% are indicated by (-). The tree resulted by Neighbor-Joining analysis (JTT+Γ4 model, pairwise deletion, bootstrap value=100) gave similar topology. The letter P added after *Arabidopsis thaliana* and *Oryza sativa* (Streptophyta) indicate plastid targeted enzymes (peroxiredoxin IIE).

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The second sequence analyzed corresponds to the subunit c of the vATPase. The subunit c is the main component of the V_0 domain of the vATPase and is highly conserved across lineages. The conservation in primary structure is related to its secondary and tertiary structure - the polypeptide is highly hydrophobic and has four membrane spanning domains, and function - it is suggested that is involved directly in H+ transport [27]. Phylogenetic analyses of this subunit revealed, with good statistical support, that *O. tauri* holds a position at the base of all green plants while *M. viride* at base of the streptophytes. In the case of *M. viride*, this result is in agreement with other studies that used different nuclear genes (Bhattacharya, Weber et al. 1998; Marin and Melkonian 1999; Karol, McCourt et al. 2001); the positioning of *M. viride* at base of the streptophytes further strengthens the confidence in the reliability of this analysis and for the placement of *O. tauri* at the base of the green plant clade.

Peroxireductases (Prx) constitute a hetereogenous class of enzymes, and represent the most recent identified group of H_2O_2-decomposing antioxidant enzymes. Their roles are multiple, they can act as antioxidans, redox sensors and modulators of pathways involved in signaling [7, 8, 29]. In land plants, Prx are well characterized and their cellular distribution is known: class II Prx, are located in the cytosol (PrxIIB-PrxIID), plastid (PrxIIE) and mitochondria (PrxIIF). The phylogenetic analysis in which several sequences from green algae were included for the first time revealed that: (i) *O. tauri*, *M. viride* and *S. dubia* are placed at the base of all green plants, basal to the node that indicate the split of streptophytes and chlorophytes; (ii) the green algal Prx are less diversified than the Prx from land plants. Moreover, their cellular distribution is puzzling, *O. tauri* Prx seems to be cytosolic, *M. viride* and *S. dubia* plastidial while the rest of the chlorophytes have short N-terminal extension when compared with cyanobacterial and protobacterial sequences, suggesting either cytosolic or mitochondrial location; (iii) compared to several alpha- and beta-proteobacteria all sequences from cyanobacteria are remotely related to green plants; (iv) The two clades that occur within *Streptophyta* suggests that several gene duplication took place within this group (or within land plants), leading to the formation of Prx with different cellular localization, i.e., cytosolic (PrxIIB-D) and plastid targeted (PrxIIE). The mitochondrial Prx (PrxIIF) from land plants are rather remotely related to the plastidial and cytosolic counterparts (data not shown), indicating either a different origin (then the Prx from land plants represent a polyphyletic group) or a divergent evolution.

Phylogenetic analyses involving two different methods, Neighbor-Joining and Mr.Bayes and three sequences, placed *M. viride* either at the base of the streptophyte clade or of all green plants while *O. tauri* was always basal to *M. viride.* These results do not reject the alternative position for *M. viride* on the tree of green plants and suggest that *Mamiellales,* the group that includes *O. tauri*, represent a lineage that diverged before the split of green plants in streptophytes and chlorophytes. Worth mentioning that recently it has been proposed that the duplication of *GapA/GapB*, genes which encode the subunit A and B of the enzyme glyceraldehyde 3-phosphate dehydrogenase, marks the origin of streptophyta [26, 32] and the most basal taxa that has *GapB* is *M. viride.* However, *O. tauri* database also contains a sequence corresponding to *GapB* gene which was not reported (entry: Ot01g01490; Borza T, unpublished data). Also, Cu-Zn superoxide dismutase was long suggested to represent a molecular characteristic (synapomorphy) of the streptophytes [5] and used to reinforce the position of *M. viride* within streptophytes [32]. Again, it seems that the *O. tauri* database was not searched for similar sequence; in fact, *O. tauri* contains two sequences with high similarity to Cu-Zn superoxide dismutase genes (entries: Ot20g00450, Ot07g01320; Borza T, unpublished data). Moreover, though it has been proposed that the *Mamiellales* might represent the most ancient group of prasinophytes [3, 21] recent phylogenetic analyses suggested that other groups of prasinophytes, such as the *Pyramidomonales* and *Prasinococcales* might have a more basal position in the evolution of the prasinophytes and chlorophytes, respectively [11, 18, 33].

Therefore, before sequences from these groups of prasinophytes will become available, cataloging various enzymes as being confined to one or the other group is questionable, while a better resolution in positioning *M. viride* and *O. tauri* on the tree of green plants might be difficult to achieve.

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MESOSTIGMA VIRIDE, OSTREOCOCCUS TAURI ŞI ORIGINEA PLANTELOR VERZI – O EVALUARE FILOGENETICĂ

(Rezumat)

Plantele verzi (*Virideplantae*) sunt reprezentate de două linii evolutive majore: *Streptophyta* and *Chlorophyta*. Clorofitele cuprind majoritatea algelor iar streptofitele sunt reprezentate de algele charofite şi de embriofite. Studiile ultrastructurale precum şi analizele filogenetice pe baza câtorva gene de provenienţa nucleară au sugerat că alga *Mesostigma viride* reprezinta cea mai ancestrală linie evolutivă desprinsă din streptofite. Alte studii filogenetice, pe baza genelor de origine plastidială şi mitocondrială, au indicat însă o origine şi mai veche pentru acest taxon: la baza tuturor plantelor verzi, înainte de apariţia divergenţei streptofite/clorofite. Genomul unei alte alge arhaice, *Ostreococcus tauri*, ce se află la baza liniei evolutive a clorofitelor, a fost secvenţiat recent aproape în totalitate. Pentru a avea o idee mai clară referitoare la poziţia filogenetică (şi taxonomică) a algei unicelulare *M. viride* şi a relaţiei ei cu *O. tauri* am decis să analizăm filogenetic trei proteine cu origine, funcţie şi localizare intracelulară diferită. Pentru reconstrucţia filogenetică s-a utilizat metoda Neighbor-Joining şi analiza Bayesiană. Studiul enzimei omega 6 acid gras desaturaza, de origine cianobacteriană şi localizare plastidială, plasează *M. viride* la baza streptofitelor, gena fiind absentă *O. tauri.* Analiza filogenetică a subunităţii c a ATPazei vacuolare, prezentă doar în eucariote, sugerează că taxonul *O. tauri* reprezintă ancestorul tuturor plantelor verzi iar *M. viride* pe cel al streptofitelor. Studiul peroxiredoxinei II, indică o posibilă origine proteobacteriană pentru această genă şi plasează atât *O. tauri* cât şi *M. viride* la baza tuturor plantelor verzi. Prin urmare, în funcţie de secvenţele alese pentru analize se pot obţine rezultate diferite ce nu exclud posibilitatea ca *M. viride* şi *O. tauri* să reprezinte linii evolutive ce au apărut înainte de separarea plantelor verzi in clorofite şi streptofite. De asemenea, acest studiu arată că poziţionarea corectă a speciilor aflate la baza arborelui filogenetic al plantelor verzi nu este posibilă fără obţinerea de cât mai multe secvenţe din grupurile bazale de alge, în special de la prasinofitele aparţinând ordinelor *Pyramidomonales* şi *Prasinococcales.*