### **Tracing the Evolution of Streptophyte Algae and their Mitochondrial Genome**

**Supplementary Material** 

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#### Table S1

Comparison of evolutionary models using cross validation and the mitochondrial data set of 9,013 positions

Models Compared	Likelihood Difference (±SD)
GTR+Γ4 vs LG+Γ4	$357.49 \pm 31.62$
$CAT+\Gamma4 \text{ vs } LG+\Gamma4$	495.77 ± 55.21
CATGTR+ $\Gamma$ 4 vs LG+ $\Gamma$ 4	$709.3 \pm 55.86$

**Fig. S1.** – Gene maps of the two klebsormidialean mitochondrial genomes sequenced in this study. Coding sequences (filled boxes) on the outside of the map are transcribed in a clockwise direction. Introns are represented by open boxes. tRNA genes are indicated by the one-letter amino acid code followed by the anticodon in parentheses (Me, elongator methionine: Mf, initiator methionine). The *Klebsorminium* genome carry two non-identical copies of *trnl*(gau); the copy located between trnG(gcc) and trnE(uuc) is more similar in sequence to its orthologues in other streptophyte mtDNAs.

**Fig. S2.** – Gene maps of the two zygnematalean mitochondrial genomes sequenced in this study. Coding sequences (filled boxes) on the outside of the map are transcribed in a clockwise direction. Introns are represented by open boxes. tRNA genes are indicated by the one-letter amino acid code followed by the anticodon in parentheses (Me, elongator methionine: Mf, initiator methionine).

**Fig. S3.** – Shared mitochondrial gene clusters between the moss *Physcomitrella* and the charophyceans *Chara/Nitella*, *Roya*, *Closterium* and *Chaetosphaeridium*. The gene order data sets used for these four pair-wise genome comparisons are presented. Each data set includes all genes/pseudogenes that are shared by *Physcomitrella* and the charophycean analyzed. Conserved clusters are denoted by boxes of different colors. The + and - signs indicate gene polarities.

**Fig. S4.** – Scenario of mitochondrial genome rearrangements during charophycean evolution and the transition to land plants. This is the best tree we inferred from a data set of 55

gene/pseudogene positions using GRAPPA and the algorithm of Caprara. The tree features the minimal number of reversals (109). Branch lengths indicate numbers of reversals.

**Fig. S5**. – Correspondence analysis of amino acid usage in the data set of 9,013 positions. Species names are abbreviated as in table 1.

**Fig. S6.** – Saturation levels of the original amino acid data set of 9,013 positions (A) and of the trimmed data set of 7,210 positions (B). The level of saturation was estimated for each data set by computing the slope of the regression line of patristic distances versus observed distances (solid line). The dashed line represents the saturation plot predicted for a data set with no substitution saturation (slope = 1). The patristic distances were inferred from branch lengths of the best tree inferred under the CATGTR+F4 model.





Figure S2

### Charales

#### Chara/Nitella

-+ +

nad4L
trnY(gua)
trnR(ucu)
trnG(ucc)
trnS(uga) mttB
trnL(uag)
trnl(cau)
atp9
rpl6
rps14 rpl5
rpl14
rpl16 rps3
rps19
rpi2 rps10
nad7
trn I (ggu) trnA(ugc)
trnS(gcu)
trnV(uac)
nad3
trnMe(cau)
atp1
rpl10
trnH(gug)
trnL(uaa)
nad5 nad4
nad2
rps7
rps12
rps12 trnE(uuc)
rps12 trnE(uuc) trnR(acg) trnl(gau)
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc)
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu)
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gac) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug)
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejV
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejU yejV rps2 trnE(aca)
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejU yejV rps2 trnF(gaa) trnC(gca)
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejV rps2 trnF(gaa) trnC(gca) rps1 atp8
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) adp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejV yejV yejV yrps2 trnF(gaa) trnC(gca) rps1 adp8 adp4
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejU yejU yejU yejV rps2 trnF(gaa) trnC(gca) rps1 atp8 atp4 cox1 sdh4
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejV yejV yejV yejV yejV yejV rps2 trnF(gaa) trnC(gca) rps1 atp8 atp4 cox1 sdh4
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejV rps2 trnF(gaa) trnC(gca) rps1 atp8 atp4 cox1 sdh4 sdh3 trnP(ugg) rms
rps12 trnE(uuc) trnR(acg) trnI(gau) trnG(gcc) atp6 nad6 trnN(guu) cox2 cox3 nad1 cob trnQ(uug) yejR yejU yejV yejV yejV yejV yejV yejV yejV yejV

rnl

# Zygnematales

#### Roya

trnY(gua) trnR(ucu) nad4L sdh4

sdh3 trnP(ugg) mttB rps4 trnG(ucc) trnS(uga) trnL(uag) trnK(uuu) trnI(cau) atp9 rps11 rps13

rpl6 rps14 rpl5 rpl16 rps3 rps19 rpl2 rps10 nad7 trnT(ggu) trnA(ugc) atp4 atp8 rps1 . trnC(gca) trnF(gaa) rps2 yejV yejU yejR trnQ(uug) cob nad1 cox3 cox2 trnN(guu) nad6 atp6 trnE(uuc) trnR(acg) trnG(gcc) rps7 rps12 nad2 nad4 nad5 trnH(gug) trnL(uaa) trnL(caa) rpl10 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnS(gcu) cox1 rns trnMe(cau) rrn5 trnMf(cau) rnl

#### Closterium

+

### Coleochaetales

#### Chaetosphaeridium

trnR(acg)
rnl
trnivit(cau)
trnMe(cau)
rns vei\/
atp6
trnE(uuc)
cox1
atp4
atp8
trnC(qca)
trnF(gaa)
rps2
yejR
trnQ(uug)
cob
nad1
cox3
trnN(quu)
nad6
trnG(gcc)
rps12
nad2
nad4 nad5
trnL(uaa)
trol (coo)
troll(cua)
trnH(gug) rpl10
trnH(gug) rpl10 nad9
trnH(gug) rpl10 nad9 atp1 trnW(cca)
trnH(gug) rpl10 nad9 atp1 trnW(cca) nad3
trnH(gug) rpl10 nad9 atp1 trnW(cca) nad3 trnV(uac)
trnH(gug) rpI10 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnD(guc)
trnH(gug) rpI10 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnS(gcu) trnA(ugc)
rp10 rp10 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnS(gcu) trnA(ugc) trnA(ugc) trnT(ggu) nad7
InnL(gag) rp110 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnS(gcu) trnA(ugc) trnT(ggu) nad7 rps10
InnL(gag) rp110 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnS(gcu) trnA(ugc) trnA(ugc) trnT(ggu) nad7 rps10 rp12
InnL(gag) rp10 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnS(gcu) trnA(ugc) trnA(ugc) trnA(ugc) trnT(ggu) nad7 rps10 rps19 rms3
tmL(cda)     tmH(gug)     rp110     nad9     atp1     tmW(cca)     nad3     tmD(guc)     tmS(gcu)     tmA(ugc)     tmA(ugc)     tmT(ggu)     nad7     rps10     rps19     rps3     rp16
InnL(Gal) rp110 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnD(guc) trnA(ugc) trnA
InnH(gug) rp110 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnD(guc) trnA(ugc) trnA(ugc) trnA(ugc) trnA(ugc) trnT(ggu) nad7 rps10 rps19 rps3 rps19 rps3 rps16 rp16 rp15 rps14 rp16
InnH(gug) rp110 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnD(guc) trnA(ugc) trnA
InnH(gug) rp110 nad9 atp1 trnW(cca) nad3 trnV(uac) trnD(guc) trnD(guc) trnA(ugc) trnA(ugc) trnA(ugc) trnA(ugc) trnT(ggu) nad7 rps10 rps19 rps3 rps14 rp16 rps13 rps11 otp0
InnH(gug) rp110 nad9 atp1 tmW(cca) nad3 tmV(uac) tmD(guc) tmC(gu) tmA(ugc) tmA(ugc) tmA(ugc) tmT(ggu) nad7 rps10 rp12 rps19 rps19 rps19 rps14 rp16 rps13 rps11 atp9 tm(cau)
InnH(gug) rp110 nad9 atp1 tmW(cca) nad3 tmV(uac) tmD(guc) tmC(gu) tmA(ugc) tmA(ugc) tmT(ggu) nad7 rps10 rp12 rps19 rps19 rps14 rp16 rps13 rps11 atp9 tmI(cau) tmA(uuu)
InnH(gug) rp110 nad9 atp1 tmW(cca) nad3 tmV(uac) tmD(guc) tmC(gu) tmA(ugc) tmA(ugc) tmA(ugc) tmT(ggu) nad7 rps10 rp12 rps19 rps19 rps14 rp16 rps13 rps11 atp9 tmA(uau) tmK(uuu) tmK(uuu) tmK(uuu)
Intractical)   trnH(gug)   rp110   nad9   atp1   trnW(cca)   nad3   trnD(guc)   trnS(gcu)   trnA(ugc)   trnT(ggu)   nad7   rps10   rp12   rps3   rp16   rps14   rp66   rps11   atp9   trnK(uuu)   trnK(uuu)   trnK(uuu)   trnG(ucc)   rps4
Intractical)   trnH(gug)   rp110   nad9   atp1   trnW(cca)   nad3   trnD(guc)   trnS(gcu)   trnA(ugc)   trnT(ggu)   nad7   rps10   rp12   rps3   rp16   rps14   rp66   rps11   atp9   trnK(uuu)   trnK(uuu)   trnG(ucc)   rps4   mtB
Intractical)   trnH(gug)   rp110   nad9   atp1   trnW(cca)   nad3   trnD(guc)   trmS(gcu)   trmA(ugc)   trmA(ugc)   trmT(ggu)   nad7   rps10   rp12   rps3   rp16   rps13   rps14   rp6   rps13   rps11   atp9   trmK(uuu)   trmG(ucc)   rps4   mtB   trnR(ucu)   trnR(ucu)
InnH(gug) rp110 nad9 atp1 tmW(cca) nad3 tmV(uac) tmD(guc) tmS(gcu) tmA(ugc) tmA(ugc) tmT(ggu) nad7 rps10 rp12 rps19 rps3 rp16 rp513 rp514 rp16 rp513 rp514 rp514 rp514 rp514 rp514 rp514 rp515 rps14 rp514 rm6(ucu) tmR(ucu) tmR(ucu) rm7(yua) nad4L
tmRL(caa)   tmH(gug)   rp110   nad9   atp1   tmW(cca)   nad3   tmV(uac)   tmD(guc)   tmM(ugc)   tmA(ugc)   tmTaggu)   nad7   rps10   rp16   rps14   rp66   rps11   atp9   tmR(ucu)   tmC(ucc)   rps4   mtB   tmR(ucu)   tmR(ucu)   tmR(ucu)   tmR(ucu)   tmR(ucu)   tmR(ucu)

rnl	+
trnT(ggu)	+
sdh3	+
sdh4	+
nad4L trnY(gua)	+
trnR(ucu)	+
mttB	+
trnG(ucc)	-
trnS(uga)	-
trnL(uag)	-
trnl(cau)	_
rps11	-
rps13 rpl6	- 1
rps14	-
rpl5	-
rps3	-
rps19	-
rpl2 rps10	2
nad7	-
trnS(acu)	-
cox3	-
cox2	-
trnV(uac)	-
nad6	-
atp6	-
trnE(uuc) trnR(acg)	-
trnl(gau)	+
trnG(gcc)	+
rps72	+
nad2	-
nad4 nad5	-
trnL(caa)	-
trnL(uaa)	+
rpl10	+
nad9	+
nad1 cob	+
trnQ(uug)	+
rps2	-
trnC(gca)	-
rps1	+
atp8 atp4	+
cox1	+
atp9	+
unvv(cca) nad3	+
trnS(gcu)	+
trnA(ugc)	+
trnMf(cau)	+
rns	+
rrn5	+

## Figure S3

sdh4



Figure S4



Figure S5



Figure S6