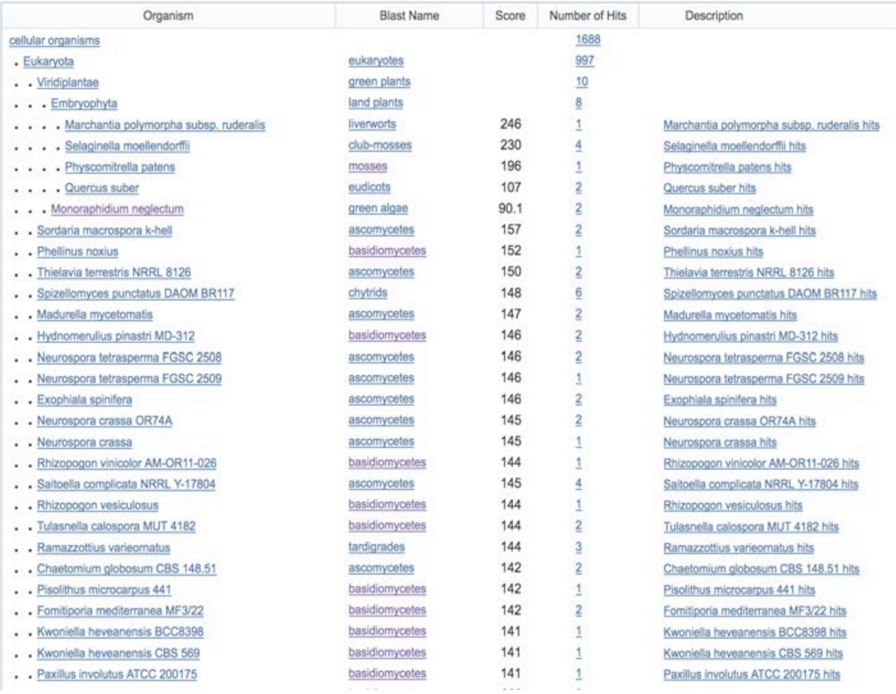
Supplementary information

for Guan et al.

Gene refashioning through innovative shifting of reading frames in mosses

a



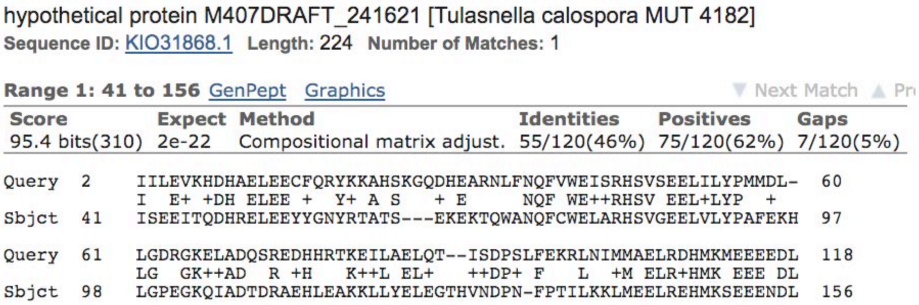
Organism	Blast Name	Score	Number of Hits	Description
cellular organisms			1688	
. Eukaryota	eukaryotes		997	
. . Viridiplantae	green plants		10	
. . . Embryophyta	land plants		8	
. . . . . Marchantia polymorpha subsp. ruderalis	liverworts	246	1	Marchantia polymorpha subsp. ruderalis hits
. . . . . Selaginella moellendorffii	club-mosses	230	4	Selaginella moellendorffii hits
. . . . . Physcomitrella patens	mosses	196	1	Physcomitrella patens hits
. . . . . Quercus suber	eudicots	107	2	Quercus suber hits
. . . . Monoraphidium neglectum	green algae	90.1	2	Monoraphidium neglectum hits
. . Sordaria macrospora k-hell	ascomycetes	157	2	Sordaria macrospora k-hell hits
. . Phellinus noxius	basidiomycetes	152	1	Phellinus noxius hits
. . Thielavia terrestris NRRL 8126	ascomycetes	150	2	Thielavia terrestris NRRL 8126 hits
. . Spizellomyces punctatus DAOM BR117	chytrids	148	6	Spizellomyces punctatus DAOM BR117 hits
. . Madurella mycetomatis	ascomycetes	147	2	Madurella mycetomatis hits
. . Hydnomerulius pinastri MD-312	basidiomycetes	146	2	Hydnomerulius pinastri MD-312 hits
. . Neurospora tetrasperma FGSC 2508	ascomycetes	146	2	Neurospora tetrasperma FGSC 2508 hits
. . Neurospora tetrasperma FGSC 2509	ascomycetes	146	1	Neurospora tetrasperma FGSC 2509 hits
. . Exophiala spinifera	ascomycetes	146	2	Exophiala spinifera hits
. . Neurospora crassa OR74A	ascomycetes	145	2	Neurospora crassa OR74A hits
. . Neurospora crassa	ascomycetes	145	1	Neurospora crassa hits
. . Rhizopogon vinicolor AM-OR11-026	basidiomycetes	144	1	Rhizopogon vinicolor AM-OR11-026 hits
. . Saitoella complicata NRRL Y-17804	ascomycetes	145	4	Saitoella complicata NRRL Y-17804 hits
. . Rhizopogon vesiculosus	basidiomycetes	144	1	Rhizopogon vesiculosus hits
. . Tulasnella calospora MUT 4182	basidiomycetes	144	2	Tulasnella calospora MUT 4182 hits
. . Ramazzottius varieornatus	tardigrades	144	3	Ramazzottius varieornatus hits
. . Chaetomium globosum CBS 148.51	ascomycetes	142	2	Chaetomium globosum CBS 148.51 hits
. . Pisolithus microcarpus 441	basidiomycetes	142	1	Pisolithus microcarpus 441 hits
. . Fomitiporia mediterranea MF3/22	basidiomycetes	142	2	Fomitiporia mediterranea MF3/22 hits
. . Kwoniella heveanensis BCC8398	basidiomycetes	141	1	Kwoniella heveanensis BCC8398 hits
. . Kwoniella heveanensis CBS 569	basidiomycetes	141	1	Kwoniella heveanensis CBS 569 hits
. . Paxillus involutus ATCC 200175	basidiomycetes	141	1	Paxillus involutus ATCC 200175 hits

b

Organism	Blast Name	Score	Number of Hits	Description
cellular organisms			1727	
. Eukaryota	eukaryotes		718	
. . Viridiplantae	green plants		18	
. . . Chlorophyceae	green algae		11	
. . . . Monoraphidium neglectum	green algae	544	6	Monoraphidium neglectum hits
. . . . Gonium pectorale	green algae	118	1	Gonium pectorale hits
. . . . Tetrabaena socialis	green algae	114	1	Tetrabaena socialis hits
. . . . Chlamydomonas reinhardtii	green algae	110	1	Chlamydomonas reinhardtii hits
. . . . Volvox carteri f. nagariensis	green algae	107	2	Volvox carteri f. nagariensis hits
. . . Selaginella moellendorffii	club-mosses	93.1	4	Selaginella moellendorffii hits
. . . Quercus suber	eudicots	91.9	2	Quercus suber hits
. . . Physcomitrella patens	mosses	83.4	1	Physcomitrella patens hits
. . Colletotrichum chlorophyti	ascomycetes	120	1	Colletotrichum chlorophyti hits
. . Coniochaeta ligniaria NRRL 30616	ascomycetes	120	1	Coniochaeta ligniaria NRRL 30616 hits
. . Colletotrichum orbiculare MAFF 240422	ascomycetes	117	1	Colletotrichum orbiculare MAFF 240422 hits
. . Allomyces macrogynus ATCC 38327	blastocladiomycetes	116	2	Allomyces macrogynus ATCC 38327 hits
. . Trichoderma reesei QM6a	ascomycetes	115	4	Trichoderma reesei QM6a hits
. . Trichoderma reesei RUT C-30	ascomycetes	115	2	Trichoderma reesei RUT C-30 hits
. . Trichoderma parareesei	ascomycetes	114	2	Trichoderma parareesei hits
. . Coniosporium apollinis CBS 100218	ascomycetes	111	2	Coniosporium apollinis CBS 100218 hits
. . Podospora anserina S mat+	ascomycetes	110	9	Podospora anserina S mat+ hits
. . Hypoxylon sp. EC38	ascomycetes	110	3	Hypoxylon sp. EC38 hits
. . Pyronema omphalodes CBS 100304	ascomycetes	110	1	Pyronema omphalodes CBS 100304 hits
. . Fusarium pseudograminearum CS3096	ascomycetes	109	2	Fusarium pseudograminearum CS3096 hits
. . Fusarium pseudograminearum CS3220	ascomycetes	109	1	Fusarium pseudograminearum CS3220 hits
. . Fusarium pseudograminearum CS3427	ascomycetes	109	1	Fusarium pseudograminearum CS3427 hits
. . Fusarium pseudograminearum CS3487	ascomycetes	109	1	Fusarium pseudograminearum CS3487 hits
. . Elaphomyces granulatus	ascomycetes	113	1	Elaphomyces granulatus hits
. . Spizellomyces punctatus DAOM BR117	chytrids	108	6	Spizellomyces punctatus DAOM BR117 hits
. . Fusarium oxysporum f. sp. melonis 26406	ascomycetes	107	7	Fusarium oxysporum f. sp. melonis 26406 hits
. . Schizosaccharomyces pombe	ascomycetes	107	2	Schizosaccharomyces pombe hits
. . Schizosaccharomyces pombe 972h-	ascomycetes	107	1	Schizosaccharomyces pombe 972h- hits

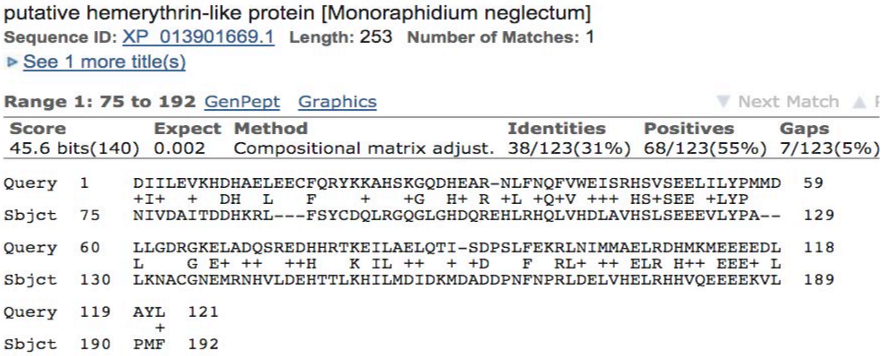
Supplementary Figure 1. (a) Partial taxonomy distribution generated from BLAST search of the NCBI non-redundant (*nr*) protein sequence database using hemerythrin from *Marchantia polymorpha* (JGI ID: Mapoly0042s0056.1). Hits from only one green alga *Monoraphidium neglectum* were found. **(b)** Partial taxonomy distribution generated from BLAST search of nr database using the *Monoraphidium* sequence identified above, indicating all green algal hits were from Chlorophyceae. In both searches, E-value = 1e-8 was used as cutoff; land plant and algal hemerythrin sequences were more similar to fungal homologs than to each other.

a



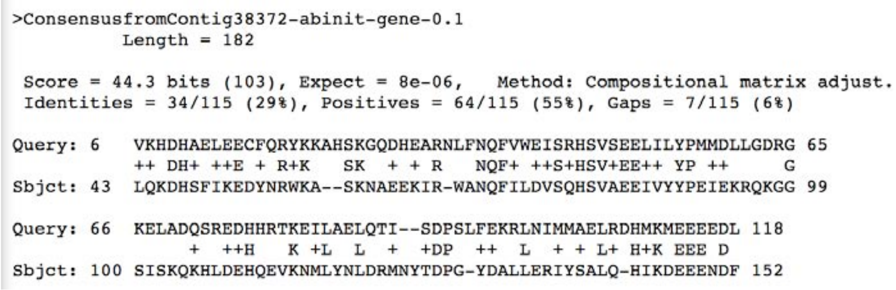
```
hypothetical protein M407DRAFT_241621 [Tulasnella calospora MUT 4182]
Sequence ID: KIO31868.1  Length: 224  Number of Matches: 1

Range 1: 41 to 156
Score           Expect  Method                        Identities     Positives      Gaps
95.4 bits(310)  2e-22   Compositional matrix adjust.  55/120(46%)    75/120(62%)    7/120(5%)

Query  2    IILEVKHDHAELEECFQRYKKAHSKGQDHEARNLFNQFVWEISRHSVSEELILYPMMDL-  60
            I  E+ +DH ELEE +  Y+ A S    + E     NQF WE++RHSV EEL+LYP   +
Sbjct  41   ISEEITQDHRELEEYYGNYRTATS---EKEKTQWANQFCWELARHSVGEELVLYPAFEKH  97

Query  61   LGDRGKELADQSREDHHRTKEILAELQT--ISDPSLFEKRLNIMMAELRDHMKMEEEEDL  118
            LG  GK++AD  R +H   K++L EL+   ++DP+ F   L  +M ELR+HMK EEE DL
Sbjct  98   LGPEGKQIADTDRAEHLEAKKLLYELEGTHVNDPN-FPTILKKLMEELREHMKSEEENDL  156
```

b



```
putative hemerythrin-like protein [Monoraphidium neglectum]
Sequence ID: XP_013901669.1  Length: 253  Number of Matches: 1

Range 1: 75 to 192
Score           Expect  Method                        Identities     Positives      Gaps
45.6 bits(140)  0.002   Compositional matrix adjust.  38/123(31%)    68/123(55%)    7/123(5%)

Query  1    DIILEVKHDHAELEECFQRYKKAHSKGQDHEAR-NLFNQFVWEISRHSVSEELILYPMMD  59
            +I+  +  DH  L   F    +   +G  H+ R +L +Q+V +++ HS+SEE +LYP
Sbjct  75   NIVDAITDDHKRL---FSYCDQLRGQGLGHDQREHLRHQLVHDLAVHSLSEEEVLYPA--  129

Query  60   LLGDRGKELADQSREDHHRTKEILAELQTI-SDPSLFEKRLNIMMAELRDHMKMEEEEDL  118
            L    G E+ ++  ++H   K IL ++  + +D   F  RL+ ++ ELR H++ EEE+ L
Sbjct  130  LKNACGNEMRNHVLDEHTTLKHILMDIDKMDADDPNFNPRLDELVHELRHHVQEEEEKVL  189

Query  119  AYL  121
             +
Sbjct  190  PMF  192
```

c



```
>ConsensusfromContig38372-abinit-gene-0.1
          Length = 182

 Score = 44.3 bits (103), Expect = 8e-06,   Method: Compositional matrix adjust.
 Identities = 34/115 (29%), Positives = 64/115 (55%), Gaps = 7/115 (6%)

Query: 6   VKHDHAELEECFQRYKKAHSKGQDHEARNLFNQFVWEISRHSVSEELILYPMMDLLGDRG 65
           ++ DH+ ++E + R+K   SK  + + R   NQF+ ++S+HSV+EE++ YP ++     G
Sbjct: 43  LQKDHSFIKEDYNRWKA--SKNAEEKIR-WANQFILDVSQHSVAEEIVYYPEIEKRQKGG 99

Query: 66  KELADQSREDHHRTKEILAELQTI--SDPSLFEKRLNIMMAELRDHMKMEEEEDL 118
                +  ++H   K +L  L  +  +DP  ++  L  + + L+ H+K EEE D
Sbjct: 100 SISKQKHLDEHQEVKNMLYNLDRMNYTDPG-YDALLERIYSALQ-HIKDEEENDF 152
```

Supplementary Figure 2. Land plant hemerythrins are more similar to fungal homologs than to those from chlorophyte green algae and glaucophytes. Figures show pairwise comparisons of the conserved HHE domain between liverwort *Marchantia polymorpha* (Mapoly0042s0093.1) and homologs from fungi, chlorophytes, and glaucophytes, respectively. Fungal and green algal sequences here are top hits from BLASTP search of nr database. **(a)** Sequence alignment between *Marchantia polymorpha* and the fungus *Tulasnella calospora*. **(b)** Sequence alignment between *Marchantia polymorpha* and chlorophyte *Monoraphidium neglectum*. **(c)** Sequence alignment between *Marchantia polymorpha* and the glaucophyte *Cyanophora paradoxa*.

a

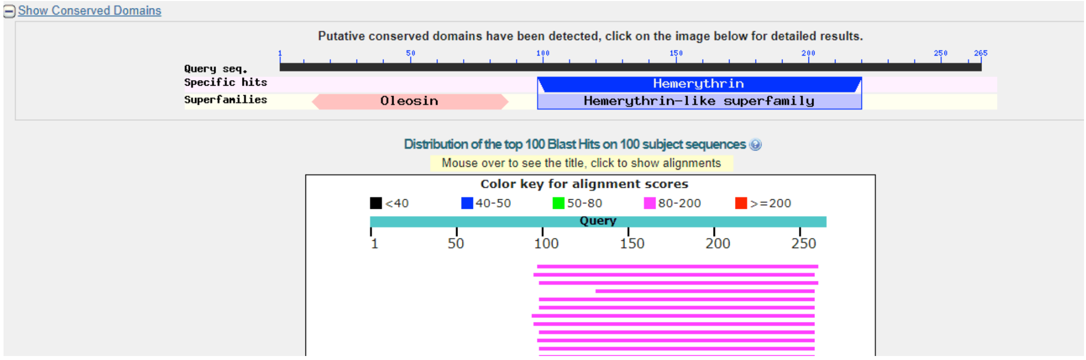
>Partial sequence of the *Isoete yunguiensis* mosaic gene derived from fusion of oleosin and *Hr*

CCTCTCGCGATGCTGGGGACGAGCCTAACCATAGGAACCCTAATTCTGGTAATATCTTCGCCGCTGTTACTCCTTTTTAGCCCAATTCTAGTACC
TCTAGGGTTTGTGCTTTTCATGGCCGCGGCAGCCTTCGCGGCTATGGTGGCAGCAGGCAACGCAGTGGCATGGATCTACCGCTACAAAAAAGG
CCGCCACCCCATGGGATCCGATAAACTTGATGCAGCAATCCACATGTTCATACCCAGAAGAGATATCATCGACCTCGTTAGGGAGGATCATGC
TAAACTGGAAGAGGATTACGGTAATTACAAGAGCGCCAGCCGTAGAGGAGACCACTACGAGGCCAGAAAATGGTTCAACCAGTTTGTGTGGG
AAATTTCCAGACATTCTGTCTCCGAAGAGCTTGTTATGTATCCCCTCCTTGATGGGCTGGGCCCAAAAGGCCGAGATTTGGCATATCAATCCCG
TGCAGATCATCACAAGATCAAGGAATTGCTCACGGAATTGCAGCACAACACTGATTCTGAGGATTTTGATTCCAGAATGGAGACCATGATGTC
CAATCTCAGAGACCATATTAAGTTGGAAGAAAAGCGCGATGGAGATCTGGCTTGTTTGCGTGATAATATGGATCAGCAGGCTCGCGAGGCTGC
CGGAGCTACATTTGCACTGGGCAAGAATCT

>Translated peptide sequence of the *Isoete yunguiensis* mosaic gene

PLAMLGTSLTIGTLILVISSPLLLLFSPILVPLGFVLFMAAAAFAAMVAAGNAVAWIYRYKKGRHPMGSDK
LDAAIHMFIPRRDIIDLVREDHAKLEEDYGNYKSASRRGDHYEARKWFNQFVWEISRHSVSEELVMYPLL
DGLGPKGRDLAYQSRADHHKIKELLTELQHNTDSEDFDSRMETMMSNLRDHIKLEEKRDGDLACLRD
NMDQQAREAAGATFALGKN

b



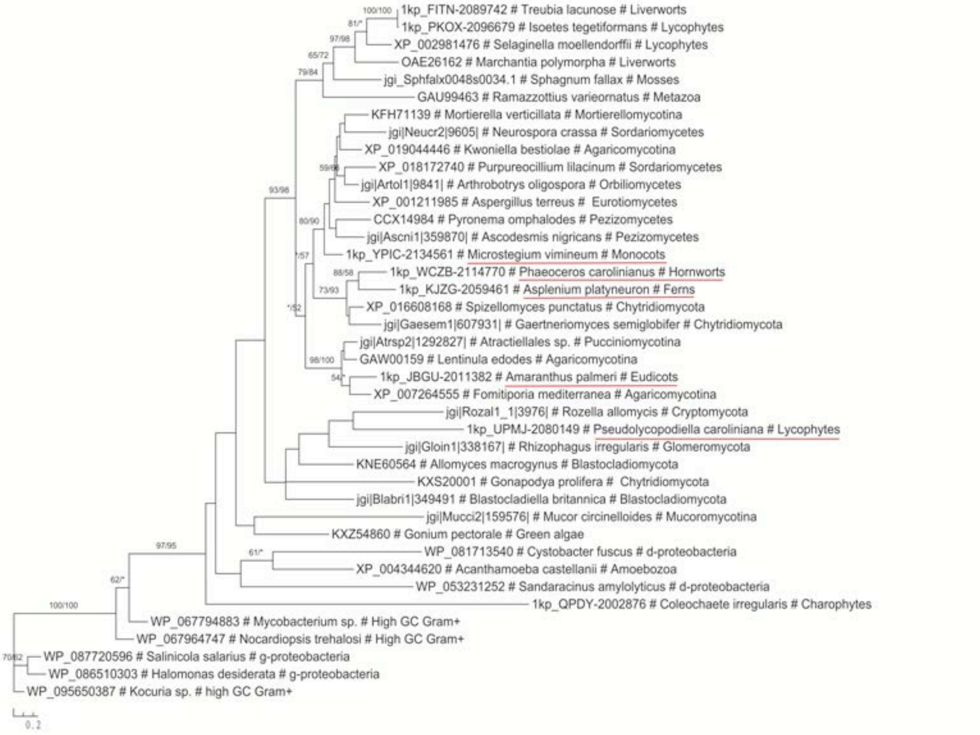
Putative conserved domains have been detected, click on the image below for detailed results.

Distribution of the top 100 Blast Hits on 100 subject sequences

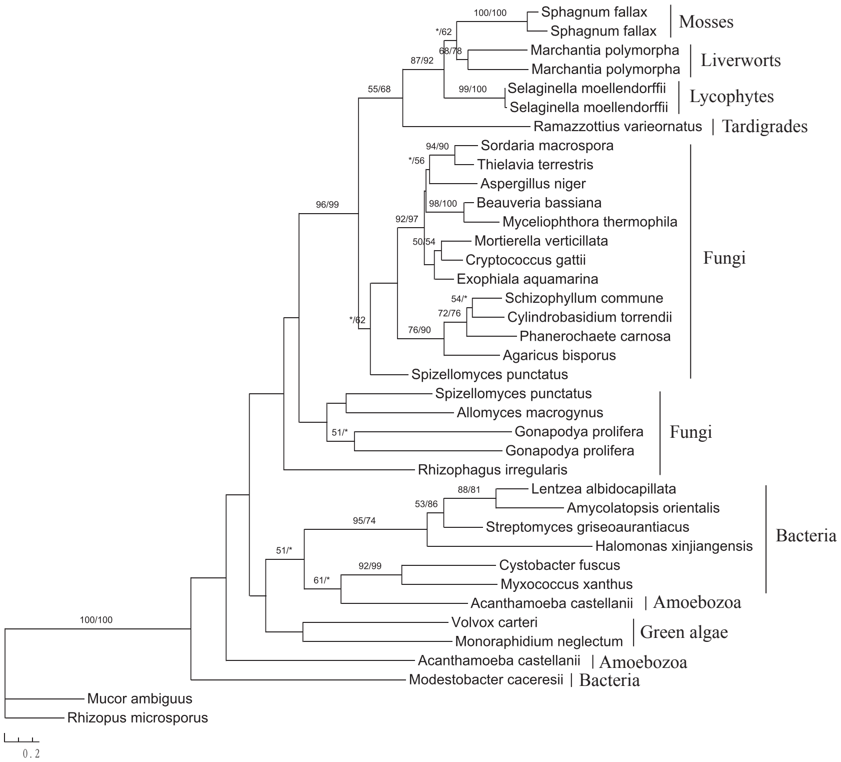
Mouse over to see the title, click to show alignments

Supplementary Figure 3. (a) Partial nucleotide sequence and translated peptide sequence of the *oleosin* and *Hr* mosaic gene in the lycophyte *Isoetes yunguiensis* (GenBank accession number MG254883). **(b)** CDD comparison with *I. yunguiensis* protein sequence, indicating fusion of oleosin and HHE domain. Although the same gene fusion was also found in the liverwort *Treubia lacunosa* (1KP ID: FITN-2089742), its nucleotide sequence shared 100% identity with PYHZ-2006808, pointing to a possibility of contamination.

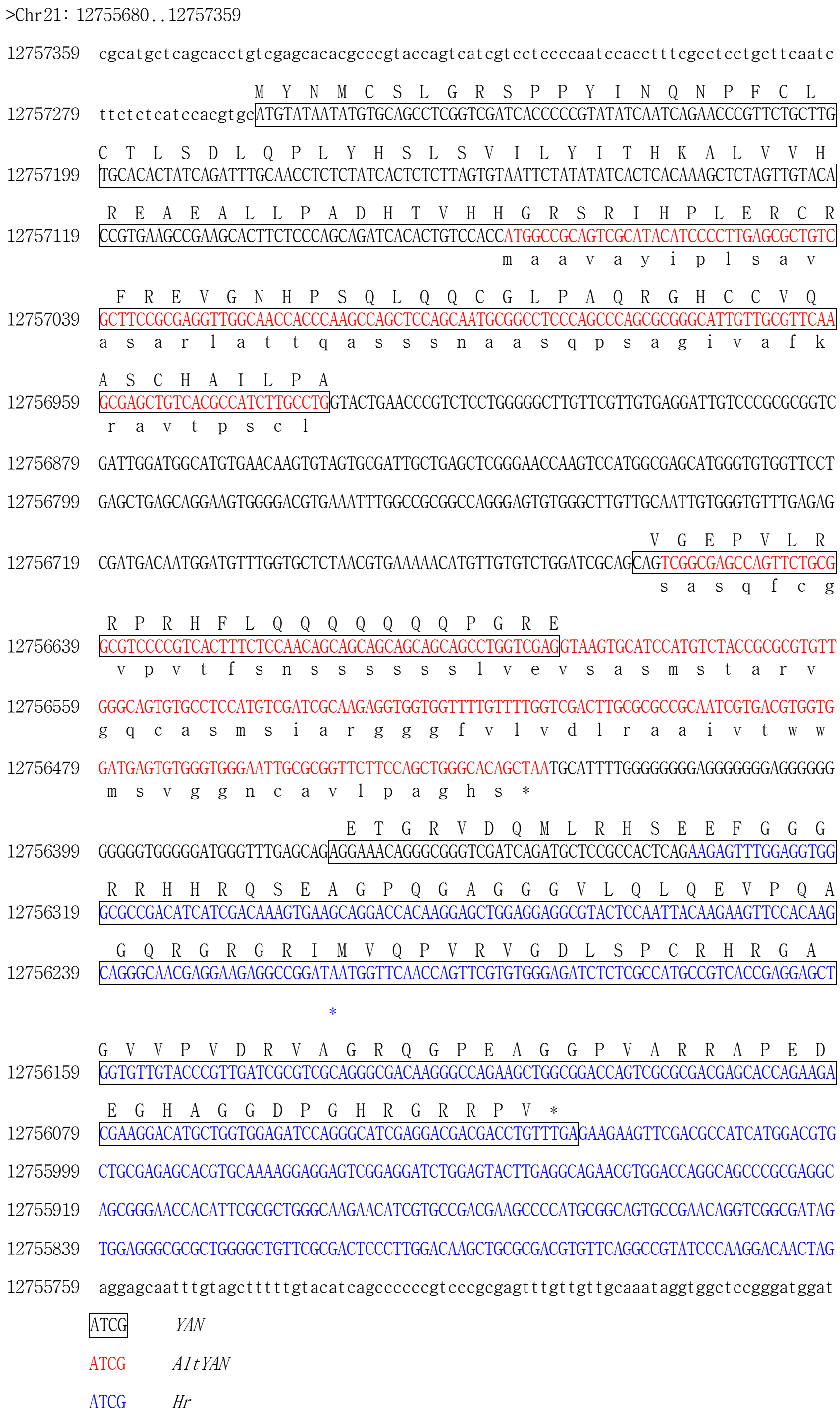
a


100/100
1kp_FITN-2089742 # Treubia lacunose # Liverworts
81/*
1kp_PKOX-2096679 # Isoetes tegetiformans # Lycophytes
97/98
XP_002981476 # Selaginella moellendorffii # Lycophytes
65/72
OAE26162 # Marchantia polymorpha # Liverworts
79/84
jgi_Sphfalx0048s0034.1 # Sphagnum fallax # Mosses
GAU99463 # Ramazzottius varieornatus # Metazoa
KFH71139 # Mortierella verticillata # Mortierellomycotina
jgi|Neucr2|9605| # Neurospora crassa # Sordariomycetes
XP_019044446 # Kwoniella bestiolae # Agaricomycotina
59/66
XP_018172740 # Purpureocillium lilacinum # Sordariomycetes
jgi|Artol1|9841| # Arthrobotrys oligospora # Orbiliomycetes
93/98
XP_001211985 # Aspergillus terreus # Eurotiomycetes
80/90
CCX14984 # Pyronema omphalodes # Pezizomycetes
jgi|Ascni1|359870| # Ascodesmis nigricans # Pezizomycetes
*/57
1kp_YPIC-2134561 # Microstegium vimineum # Monocots
88/58
1kp_WCZB-2114770 # Phaeoceros carolinianus # Hornworts
73/93
1kp_KJZG-2059461 # Asplenium platyneuron # Ferns
*/52
XP_016608168 # Spizellomyces punctatus # Chytridiomycota
jgi|Gaesem1|607931| # Gaertneriomyces semiglobifer # Chytridiomycota
jgi|Atrsp2|1292827| # Atractiellales sp. # Pucciniomycotina
98/100
GAW00159 # Lentinula edodes # Agaricomycotina
54/*
1kp_JBGU-2011382 # Amaranthus palmeri # Eudicots
XP_007264555 # Fomitiporia mediterranea # Agaricomycotina
jgi|Rozal1_1|3976| # Rozella allomycis # Cryptomycota
1kp_UPMJ-2080149 # Pseudolycopodiella caroliniana # Lycophytes
jgi|Gloin1|338167| # Rhizophagus irregularis # Glomeromycota
KNE60564 # Allomyces macrogynus # Blastocladiomycota
KXS20001 # Gonapodya prolifera # Chytridiomycota
jgi|Blabri1|349491 # Blastocladiella britannica # Blastocladiomycota
jgi|Mucci2|159576| # Mucor circinelloides # Mucoromycotina
KXZ54860 # Gonium pectorale # Green algae
97/95
61/*
WP_081713540 # Cystobacter fuscus # d-proteobacteria
XP_004344620 # Acanthamoeba castellanii # Amoebozoa
62/*
WP_053231252 # Sandaracinus amylolyticus # d-proteobacteria
100/100
1kp_QPDY-2002876 # Coleochaete irregularis # Charophytes
WP_067794883 # Mycobacterium sp. # High GC Gram+
WP_067964747 # Nocardiopsis trehalosi # High GC Gram+
70/62
WP_087720596 # Salinicola salarius # g-proteobacteria
WP_086510303 # Halomonas desiderata # g-proteobacteria
WP_095650387 # Kocuria sp. # high GC Gram+
0.2

b

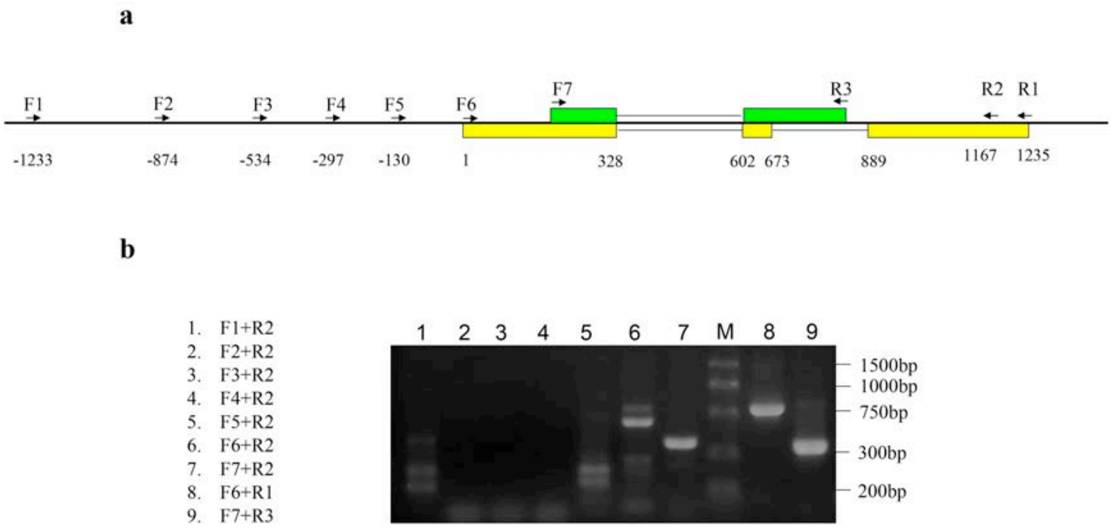

100/100
Sphagnum fallax
*/62
Sphagnum fallax
Mosses
68/78
Marchantia polymorpha
87/92
Marchantia polymorpha
Liverworts
55/68
99/100
Selaginella moellendorffii
Selaginella moellendorffii
Lycophytes
Ramazzottius varieornatus
Tardigrades
94/90
Sordaria macrospora
*/56
Thielavia terrestris
Aspergillus niger
96/99
98/100
Beauveria bassiana
92/97
Myceliophthora thermophila
50/54
Mortierella verticillata
Cryptococcus gattii
Exophiala aquamarina
Fungi
54/*
Schizophyllum commune
72/76
Cylindrobasidium torrendii
*/62
76/90
Phanerochaete carnosa
Agaricus bisporus
Spizellomyces punctatus
Spizellomyces punctatus
Allomyces macrogynus
51/*
Gonapodya prolifera
Gonapodya prolifera
Fungi
Rhizophagus irregularis
88/81
Lentzea albidocapillata
53/86
Amycolatopsis orientalis
95/74
Streptomyces griseoaurantiacus
Halomonas xinjiangensis
Bacteria
51/*
92/99
Cystobacter fuscus
61/*
Myxococcus xanthus
Acanthamoeba castellanii
Amoebozoa
100/100
Volvox carteri
Monoraphidium neglectum
Green algae
Acanthamoeba castellanii
Amoebozoa
Modestobacter caceresii
Bacteria
Mucor ambiguus
Rhizopus microsporus
0.2

Supplementary Figure 4. Molecular phylogeny of hemerythrin (Hr) protein sequences. LG and JTT substitution models were used in maximum and distance analyses, respectively, and four-gamma rate categories plus invariant sites were used in the analyses. Numbers above branches show bootstrap values from maximum likelihood and distance analyses, respectively. Asterisks indicate values lower than 50%. Nodes without values indicate that bootstrap support values from both analyses are lower than 50%. Taxonomic affiliations are after species names. **(a)** Phylogenetic analyses with a larger taxonomic sampling, including multiple angiosperm and seedless vascular plant sequences from 1KP (underlined in red). **(b)** Phylogenetic analyses with sequences of known scaffold information, mostly from genome sequence projects. The sequence of glaucophyte *Cyanophora paradoxa* was removed from the analyses because of its much shorter length. Inclusion of the *Cyanophora* sequence provided a similar topology, with much lower branch support values.



```
>Chr21: 12755680..12757359

12757359  cgcatgctcagcacctgtcgagcacacgcccgtaccagtcatcgtcctccccaatccacctttcgcctcctgcttcaatc

                           M  Y  N  M  C  S  L  G  R  S  P  P  Y  I  N  Q  N  P  F  C  L
12757279  ttctctcatccacgtgcATGTATAATATGTGCAGCCTCGGTCGATCACCCCCGTATATCAATCAGAACCCGTTCTGCTTG

          C  T  L  S  D  L  Q  P  L  Y  H  S  L  S  V  I  L  Y  I  T  H  K  A  L  V  V  H
12757199  TGCACACTATCAGATTTGCAACCTCTCTATCACTCTCTTAGTGTAATTCTATATATCACTCACAAAGCTCTAGTTGTACA

           R  E  A  E  A  L  L  P  A  D  H  T  V  H  H  G  R  S  R  I  H  P  L  E  R  C  R
12757119  CCGTGAAGCCGAAGCACTTCTCCCAGCAGATCACACTGTCCACCATGGCCGCAGTCGCATACATCCCCTTGAGCGCTGTC
                                                      m  a  a  v  a  y  i  p  l  s  a  v

            F  R  E  V  G  N  H  P  S  Q  L  Q  Q  C  G  L  P  A  Q  R  G  H  C  C  V  Q
12757039  GCTTCCGCGAGGTTGGCAACCACCCAAGCCAGCTCCAGCAATGCGGCCTCCCAGCCCAGCGCGGGCATTGTTGCGTTCAA
          a  s  a  r  l  a  t  t  q  a  s  s  s  n  a  a  s  q  p  s  a  g  i  v  a  f  k

          A  S  C  H  A  I  L  P  A
12756959  GCGAGCTGTCACGCCATCTTGCCTGGTACTGAACCCGTCTCCTGGGGGCTTGTTCGTTGTGAGGATTGTCCCGCGCGGTC
           r  a  v  t  p  s  c  l

12756879  GATTGGATGGCATGTGAACAAGTGTAGTGCGATTGCTGAGCTCGGGAACCAAGTCCATGGCGAGCATGGGTGTGGTTCCT

12756799  GAGCTGAGCAGGAAGTGGGGACGTGAAATTTGGCCGCGGCCAGGGAGTGTGGGCTTGTTGCAATTGTGGGTGTTTGAGAG

                                                                      V  G  E  P  V  L  R
12756719  CGATGACAATGGATGTTTGGTGCTCTAACGTGAAAAACATGTTGTGTCTGGATCGCAGCAGTCGGCGAGCCAGTTCTGCG
                                                                       s  a  s  q  f  c  g

           R  P  R  H  F  L  Q  Q  Q  Q  Q  Q  Q  P  G  R  E
12756639  GCGTCCCCGTCACTTTCTCCAACAGCAGCAGCAGCAGCAGCCTGGTCGAGGTAAGTGCATCCATGTCTACCGCGCGTGTT
            v  p  v  t  f  s  n  s  s  s  s  s  s  l  v  e  v  s  a  s  m  s  t  a  r  v

12756559  GGGCAGTGTGCCTCCATGTCGATCGCAAGAGGTGGTGGTTTTGTTTTGGTCGACTTGCGCGCCGCAATCGTGACGTGGTG
          g  q  c  a  s  m  s  i  a  r  g  g  g  f  v  l  v  d  l  r  a  a  i  v  t  w  w

12756479  GATGAGTGTGGGTGGGAATTGCGCGGTTCTTCCAGCTGGGCACAGCTAATGCATTTTGGGGGGGAGGGGGGGAGGGGGG
           m  s  v  g  g  n  c  a  v  l  p  a  g  h  s  *

                                     E  T  G  R  V  D  Q  M  L  R  H  S  E  E  F  G  G  G
12756399  GGGGGTGGGGGATGGGTTTGAGCAGAGGAAACAGGGCGGGTCGATCAGATGCTCCGCCACTCAGAAGAGTTTGGAGGTGG

           R  R  H  H  R  Q  S  E  A  G  P  Q  G  A  G  G  G  V  L  Q  L  Q  E  V  P  Q  A
12756319  GCGCCGACATCATCGACAAAGTGAAGCAGGACCACAAGGAGCTGGAGGAGGCGTACTCCAATTACAAGAAGTTCCACAAG

            G  Q  R  G  R  G  R  I  M  V  Q  P  V  R  V  G  D  L  S  P  C  R  H  R  G  A
12756239  CAGGGCAACGAGGAAGAGGCCGGATAATGGTTCAACCAGTTCGTGTGGGAGATCTCTCGCCATGCCGTCACCGAGGAGCT

                                   *

          G  V  V  P  V  D  R  V  A  G  R  Q  G  P  E  A  G  G  P  V  A  R  R  A  P  E  D
12756159  GGTGTTGTACCCGTTGATCGCGTCGCAGGGCGACAAGGGCCAGAAGCTGGCGGACCAGTCGCGCGACGAGCACCAGAAGA

           E  G  H  A  G  G  D  P  G  H  R  G  R  R  P  V  *
12756079  CGAAGGACATGCTGGTGGAGATCCAGGGCATCGAGGACGACGACCTGTTTGAGAAGAAGTTCGACGCCATCATGGACGTG

12755999  CTGCGAGAGCACGTGCAAAAGGAGGAGTCGGAGGATCTGGAGTACTTGAGGCAGAACGTGGACCAGGCAGCCCGCGAGGC

12755919  AGCGGGAACCACATTCGCGCTGGGCAAGAACATCGTGCCGACGAAGCCCCATGCGGCAGTGCCGAACAGGTCGGCGATAG

12755839  TGGAGGGCGCGCTGGGGCTGTTCGCGACTCCCTTGGACAAGCTGCGCGACGTGTTCAGGCCGTATCCCAAGGACAACTAG

12755759  aggagcaatttgtagctttttgtacatcagccccccgtcccgcgagtttgttgttgcaaataggtggctccgggatggat

         ATCG     YAN

         ATCG     A1tYAN

         ATCG     Hr
```

Supplementary Figure 5. Sequences of *YAN*, *AltYAN*, and *Hr*. Black boxes indicate exons of *YAN*. Red letters show exons of *AltYAN*. Blue letters show the pseudogenized *Hr* region. Capital letters above the nucleotide sequences indicate the amino acids encoded by *YAN*, while lowercase letters below indicate those encoded by *AltYAN*.



Supplementary Figure 6. RT-PCR confirmation of *YAN*, *AltYAN*, and *Hr*. (**a**) Schematic diagram of primers. To confirm the mRNA completeness of this locus, seven forward primers were designed, with F1-F5 annealing to the upstream and F6-F7 annealing to exons. Each forward primer cooperated with a reverse primer R2 for RT-PCR reactions. To further confirm the CDS sequences of *YAN* and *AltYAN*, two primer pairs, (F6, R1) and (F7, R3), were used to amplify the CDSs of *YAN* and *AltYAN*, respectively. Amplified CDSs were then sequenced. (**b**) Gel result of RT-PCR. F1-F5 cooperating with R2 failed to amplify the appropriate product, but F6 and F7 cooperating with R2 could amplify the appropriate product. The lengths of *YAN* and *AltYAN* CDS sequences were correct.

a

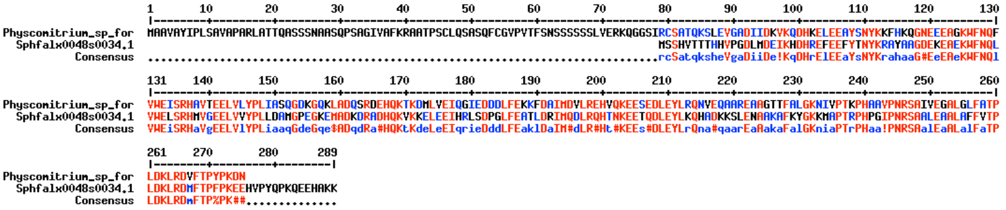
>*Physcomitrium sp.* *YAN* and *Hr* annotation

```
                 M  Y  N  M  C  S  L  G  R  S  P  P  Y  I  N  Q  N  S  F  C  L
CTCATCCACGTGCATGTATAATATGTGCAGCCTCGGTCGATCACCCCCGTACATCAACCAGAACTCGTTCTGCTT
  C  T  L S  D  L Q  P  L  Y H  S  L  S  V  I  L Y I  T H  K  A  L  V
GTGCACACTATCAGATTTGCAACCTCTCTATCACTCGCTCAGTGTAATTCTATATATCACTCACAAAGCTCTAGT
  V H  R  E  A  E  A  L  L  P  A  D H  T  V H  H  G  R  S  R I  H  P
TGTACACCGTGAAGCCGAAGCACTTCTCCCAGCAGATCACACTGTCCACCATGGCCGCAGTCGCATACATCC
                                                     m  a   a  v  a  y  i
  L  E  R C  R  S  R  E  V  G N  H  P  S  Q  L  Q  Q C  G  L  P  A  Q
CCTTGAGCGCTGTCGCTCCCGCGAGGTTGGCAACCACCCAAGCCAGCTCCAGCAATGCGGCCTCCCAGCCCA
p  l  s  a  v  a  p  a  r  l  a  t  t  q  a  s  s  s  n  a  a  s  q  p
  C  G  H C  C V  Q  A  S  C  H A  I  L P
GTGCGGGCATTGTTGCGTTCAAGCGAGCTGCCACGCCATCTTGCCTG
s  a  g  i  v  a  f  k  r  a  a  t  p  s  c  l

A  V  G  E  P  V  L R  R  P  R  H  F L  Q  Q  Q  Q Q  Q  Q  P  G  R
CAGTCGGCGAGCCAGTTCTGCGGCGTCCCCGTCACTTTCTCCAACAGCAGCAGCAGCAGCAGCCTGGTCGAG
 q  s  a  s  q  f  c  g  v  p  v  t  f  s  n  s  s  s  s  s  s  l  v  e

E  E  T  G  R  V D  Q  M  L  R H  S  E  E F  G  G  G  R  R  H H  R
AGGAAACAGGGCGGGTCGATCAGATGCTCCGCCACTCAGAAGAGTTTGGAGGTGGGCGCCGACATCATCGAC
 r  k q  g  g  s i  r  c  s a  t  q  k  s l  e  v  g a  d  i i  d
Q  S  E  A  G  P  Q G  A  G  G  G  V  L Q  L Q  E  V  P  Q  A  G  Q
AAAGTGAAGCAGGACCACAAGGAGCTGGAGGAGGCGTACTCCAATTACAAGAAGTTCCACAAGCAGGGCAA
 k  v  k  q  d  h  k  e  l  e  e  a  y  s  n y  k  k  f  h  k  q  g  n
 R  G  R  G  R  K  M  V Q  P  V  R V  G  D  L A  P  C  R  H  R  G  A
CGAGGAAGAGGCCGGAAAATGGTTCAACCAGTTCGTGTGGGAGATCTCGCGCCATGCCGTCACCGAGGAGCT
 e  e  e  a  g  k  w  f  n  q  f  v  w  e  i  s  r  h  a  v  t  e  e  l
 G V  V  P V  D  R V  A  G  R  Q  G  P  E  A  G  G  P  V A  R  R  A
GGTGTTGTACCCGTTGATCGCGTCGCAGGGCGACAAGGGCCAGAAGCTGGCGGACCAGTCGCGCGACGAGC
 v  l  y  p  l  i  a  s  q  g  d  k  g  q  k  l  a  d  q  s  r  d  e
 P  E  D  E  G H  A G  G  D  P  G  H R  G  R  R  P  V  *
ACCAGAAGACGAAGGACATGCTGGTGGAGATCCAGGGCATCGAGGACGACGACCTGTTTGAGAAGAAGTTC
 h  q  k  t  k  d  m  l  v  e  i  q  g  i  e  d  d  d  l  f  e  k  k  f
GACGCAATCATGGACGTGCTGCGAGAGCACGTGCAAAAGGAGGAGTCGGAGGATCTGGAGTACTTGAGGCA
 d  a  i  m  d  v  l  r  e  h  v  q  k  e  e  s  e  d  l  e  y  l  r  q
GAACGTGGAACAGGCAGCCCGCGAGGCAGCGGGAACCACATTCGCGCTGGGCAAGAACATCGTGCCGACGA
 n  v  e  q  a  a  r  e  a  a  g  t  t  f  a  l  g  k  n  i  v  p  t
AGCCCCATGCGGCAGTGCCGAACAGGTCGGCGATAGTGGAGGGCGCCCTGGGGCTGTTCGCGACTCCCTTGG
k  p  h  a  a  v  p  n  r  s  a  i  v  e  g  a  l  g  l  f  a  t  p  l
ACAAGCTGCGCGACGTGTTCACGCCGTATCCCAAGGACAACTAGAGGAGCAATTTGTAGCTTTTTGTACATCA
d  k  l  r  d  v  f  t  p  y  p  k  d  n  *
```

ATCG *YAN*

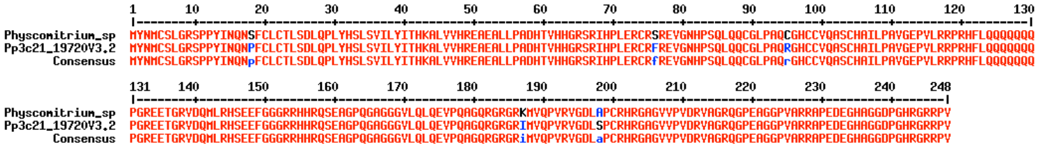
ATCG *Hr*

b



```
                      1       10        20        30        40        50        60        70        80        90        100       110       120       130
                      |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Physcomitrium_sp_for  MAAVAYIPLSAVAPARLATTQASSSNAASQPSAGIVAFKRAATPSCLQSASQFCGVPVTFSNSSSSSSLVERKQGGSIRCSATQKSLEVGADIIDKVKQDHKELEEAYSNYKKFHKQGNEEEAGKWFNQF
  Sphfalx0048s0034.1                                                                                 MSSHVTTTHHVPGDLMDEIKHDHREFEEFYTNYKRAYAAGDEKEAEKWFNQL
           Consensus  ..............................................................................rcSatqksheVgaDiiDe!KqDHrElEEaYsNYKrahaaG#EeEAeKWFNQl

                      131     140       150       160       170       180       190       200       210       220       230       240       250       260
                      |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Physcomitrium_sp_for  VWEISRHAVTEELVLYPLIASQGDKGQKLADQSRDEHQKTKDMLVEIQGIEDDDLFEKKFDAIMDVLREHVQKEESEDLEYLRQNVEQAAREAAGTTFALGKNIVPTKPHAAVPNRSAIVEGALGLFATP
  Sphfalx0048s0034.1  VWELSRHMVGEELVVYPLLDAMGPEGKEMADKDRADHQKVKKELEEIHRLSDPGLFEATLDRIMQDLRQHTNKEETQDLEYLKQHADKKSLENAAKAFKYGKKMAPTRPHPGIPNRSAALEAALAFFVTP
           Consensus  VWEiSRHaVgEELVlYPLiaaqGdeGqe$ADqdRa#HQKtKdeLeEIqrieDddLFEaklDaIM#dLR#Ht#KEEs#DLEYLrQna#qaarEaAakaFalGKniaPTrPHaa!PNRSAalEaALalFaTP

                      261     270       280      289
                      |--------+---------+--------|
Physcomitrium_sp_for  LDKLRDVFTPYPKDN
  Sphfalx0048s0034.1  LDKLRDMFTPFPKEEHVPYQPKQEEHAKK
           Consensus  LDKLRDmFTP%PK##..............
```

c

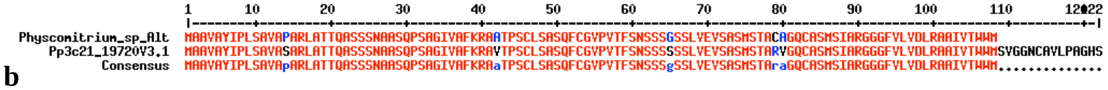


```
                    1       10        20        30        40        50        60        70        80        90        100       110       120       130
                    |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Physcomitrium_sp    MYNMCSLGRSPPYINQNSFCLCTLSDLQPLYHSLSVILYITHKALVVHREAEALLPADHTVHHGRSRIHPLERCRSREVGNHPSQLQQCGLPAQCGHCCVQASCHAILPAVGEPVLRRPRHFLQQQQQQQ
Pp3c21_19720V3.2    MYNMCSLGRSPPYINQNPFCLCTLSDLQPLYHSLSVILYITHKALVVHREAEALLPADHTVHHGRSRIHPLERCRFREVGNHPSQLQQCGLPAQRGHCCVQASCHAILPAVGEPVLRRPRHFLQQQQQQQ
       Consensus    MYNMCSLGRSPPYINQNpFCLCTLSDLQPLYHSLSVILYITHKALVVHREAEALLPADHTVHHGRSRIHPLERCRfREVGNHPSQLQQCGLPAQrGHCCVQASCHAILPAVGEPVLRRPRHFLQQQQQQQ

                    131     140       150       160       170       180       190       200       210       220       230       240     248
                    |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+-------|
Physcomitrium_sp    PGREETGRVDQMLRHSEEFGGGRRHHRQSEAGPQGAGGGVLQLQEVPQAGQRGRGRKMVQPVRVGDLAPCRHRGAGVVPVDRVAGRQGPEAGGPVARRAPEDEGHAGGDPGHRGRRPV
Pp3c21_19720V3.2    PGREETGRVDQMLRHSEEFGGGRRHHRQSEAGPQGAGGGVLQLQEVPQAGQRGRGRIMVQPVRVGDLSPCRHRGAGVVPVDRVAGRQGPEAGGPVARRAPEDEGHAGGDPGHRGRRPV
       Consensus    PGREETGRVDQMLRHSEEFGGGRRHHRQSEAGPQGAGGGVLQLQEVPQAGQRGRGRiMVQPVRVGDLaPCRHRGAGVVPVDRVAGRQGPEAGGPVARRAPEDEGHAGGDPGHRGRRPV
```

Supplementary Figure 7. **(a)** Sequence and annotation of RT-PCR product of the HHE region in *Physcomitrium sp*. (GenBank accession number MG254884). Living materials were collected from Xinping, Yunnan, China. Amino acids encoded by the *Hr* gene are shown by lower case characters below the nucleotide sequence. Upper case characters above the nucleotide sequence indicate amino acids encoded by *YAN*. Boxes show exons of *YAN* (GenBank accession number MG254882). **(b)** Alignment of putative Hr protein sequences from *Physcomitrium sp*. and *Sphagnum fallax*. **(c)** Alignment of putative YAN protein sequences from *Physcomitrium sp*. and *Physcomitrella patens*.

a

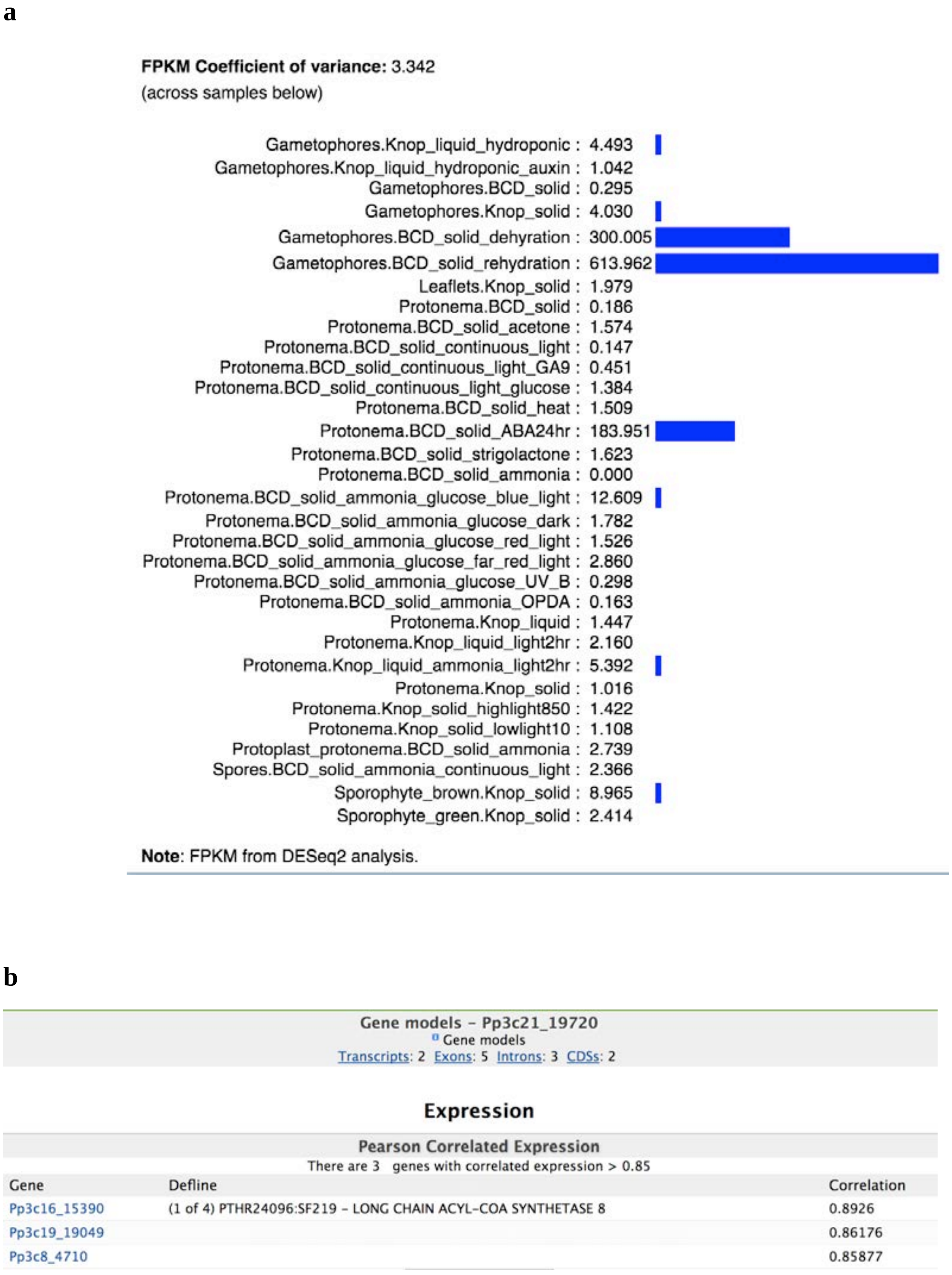
```
>Physcomitrium sp. AltYAN annotation
ATGGCCGCAGTCGCATACATCCCCTTGAGCGCTGTCGCTCCCGCGAGGTTG
 M   A   A   V   A   Y   I   P   L   S   A   V   A   P   A   R   L
GCAACCACCCAAGCCAGCTCCAGCAATGCGGCCTCCCAGCCCAGTGCGGGC
  A   T   T   Q   A   S   S   S   N   A   A   S   Q   P   S   A   G
ATTGTTGCGTTCAAGCGAGCTGCCACGCCATCTTGCCTG
 I   V   A   F   K   R   A   A   T   P   S   C   L

TCGGCGAGCCAGTTCTGCGGCGTCCCCGTCACTTTCTCCAACAGCAGCAGC
  S   A   S   Q   F   C   G   V   P   V   T   F   S   N   S   S   S
GGCAGCAGCCTGGTCGAGGTAAGTGCATCCATGTCCACCGCGTGTGCTGGG
  G   S   S   L   V   E   V   S   A   S   M   S   T   A   C   A   G
CAGTGTGCCTCCATGTCGATCGCCAGAGGTGGTGGTTTTGTTTTGGTCGAC
  Q   C   A   S   M   S   I   A   R   G   G   G   F   V   L   V   D
TTGCGCGCCGCAATCGTGACGTGGTGGATGA……
 L   R   A   A   I   V   T   W   W   M
```



```
                    1       10        20        30        40        50        60        70        80        90        100       110       12122
                    |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+-|
Physcomitrium_sp_Alt MAAVAYIPLSAVAPARLATTQASSSNAASQPSAGIVAFKRAATPSCLSASQFCGVPVTFSNSSSGSSLVEVSASMSTACAGQCASMSIARGGGFVLVDLRAAIVTWWM
    Pp3c21_19720V3.1 MAAVAYIPLSAVASARLATTQASSSNAASQPSAGIVAFKRAVTPSCLSASQFCGVPVTFSNSSSSSSLVEVSASMSTARVGQCASMSIARGGGFVLVDLRAAIVTWWMSVGGNCAVLPAGHS
           Consensus MAAVAYIPLSAVApARLATTQASSSNAASQPSAGIVAFKRAaTPSCLSASQFCGVPVTFSNSSSgSSLVEVSASMSTAraGQCASMSIARGGGFVLVDLRAAIVTWWM.............
```

b

Supplementary Figure 8. **(a)** Partial sequence and annotation of RT-PCR product of *AltYAN* in *Physcomitrium sp*. (GenBank accession number: MG254881). Primers specific to the *AltYAN* region were used for RT-PCR reactions. Living materials were collected from Xinping, Yunnan, China. Amino acids encoded by *AltYAN* are shown below the nucleotide sequence. **(b)** Alignment of putative AltYAN protein sequences from *Physcomitrium sp*. and *Physcomitrella patens*.



a

FPKM Coefficient of variance: 3.342
(across samples below)

Gametophores.Knop_liquid_hydroponic :	4.493
Gametophores.Knop_liquid_hydroponic_auxin :	1.042
Gametophores.BCD_solid :	0.295
Gametophores.Knop_solid :	4.030
Gametophores.BCD_solid_dehyration :	300.005
Gametophores.BCD_solid_rehydration :	613.962
Leaflets.Knop_solid :	1.979
Protonema.BCD_solid :	0.186
Protonema.BCD_solid_acetone :	1.574
Protonema.BCD_solid_continuous_light :	0.147
Protonema.BCD_solid_continuous_light_GA9 :	0.451
Protonema.BCD_solid_continuous_light_glucose :	1.384
Protonema.BCD_solid_heat :	1.509
Protonema.BCD_solid_ABA24hr :	183.951
Protonema.BCD_solid_strigolactone :	1.623
Protonema.BCD_solid_ammonia :	0.000
Protonema.BCD_solid_ammonia_glucose_blue_light :	12.609
Protonema.BCD_solid_ammonia_glucose_dark :	1.782
Protonema.BCD_solid_ammonia_glucose_red_light :	1.526
Protonema.BCD_solid_ammonia_glucose_far_red_light :	2.860
Protonema.BCD_solid_ammonia_glucose_UV_B :	0.298
Protonema.BCD_solid_ammonia_OPDA :	0.163
Protonema.Knop_liquid :	1.447
Protonema.Knop_liquid_light2hr :	2.160
Protonema.Knop_liquid_ammonia_light2hr :	5.392
Protonema.Knop_solid :	1.016
Protonema.Knop_solid_highlight850 :	1.422
Protonema.Knop_solid_lowlight10 :	1.108
Protoplast_protonema.BCD_solid_ammonia :	2.739
Spores.BCD_solid_ammonia_continuous_light :	2.366
Sporophyte_brown.Knop_solid :	8.965
Sporophyte_green.Knop_solid :	2.414

Note: FPKM from DESeq2 analysis.

b

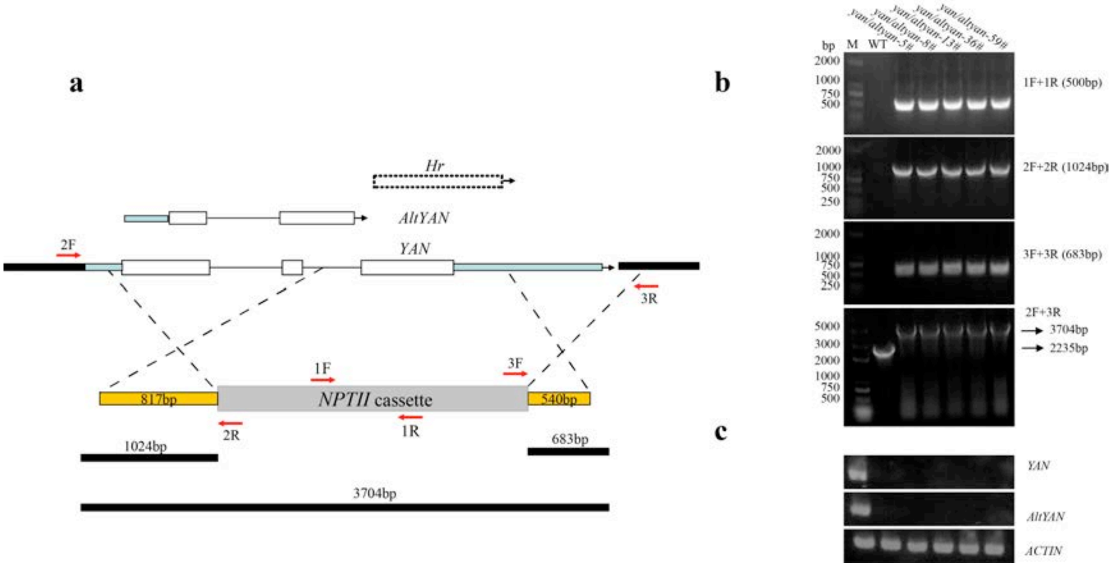
Gene models – Pp3c21_19720
Gene models
Transcripts: 2 Exons: 5 Introns: 3 CDSs: 2

Expression

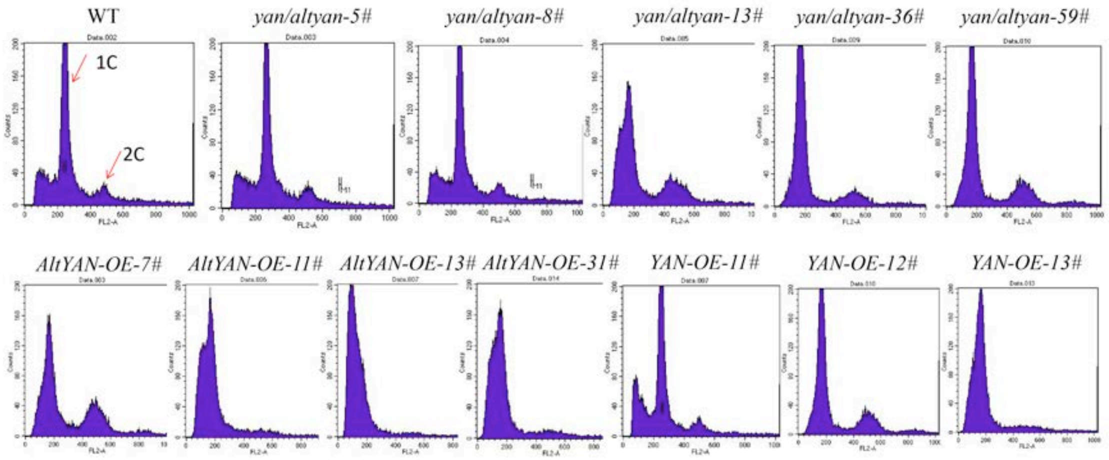
Pearson Correlated Expression
There are 3 genes with correlated expression > 0.85

Gene	Defline	Correlation
Pp3c16_15390	(1 of 4) PTHR24096:SF219 – LONG CHAIN ACYL-COA SYNTHETASE 8	0.8926
Pp3c19_19049		0.86176
Pp3c8_4710		0.85877

Supplementary Figure 9. Transcription data of *YAN/AltYAN* from Phytozome (https://phytozome.jgi.doe.gov/phytomine/report.do?id=237468538). **(a)** *YAN/AltYAN* is strongly induced by both dehydration and rehydration. **(b)** *YAN/AltYAN* is co-expressed with multiple other genes, including long-chain acyl-CoA synthetase 8 (*LACS 8*).



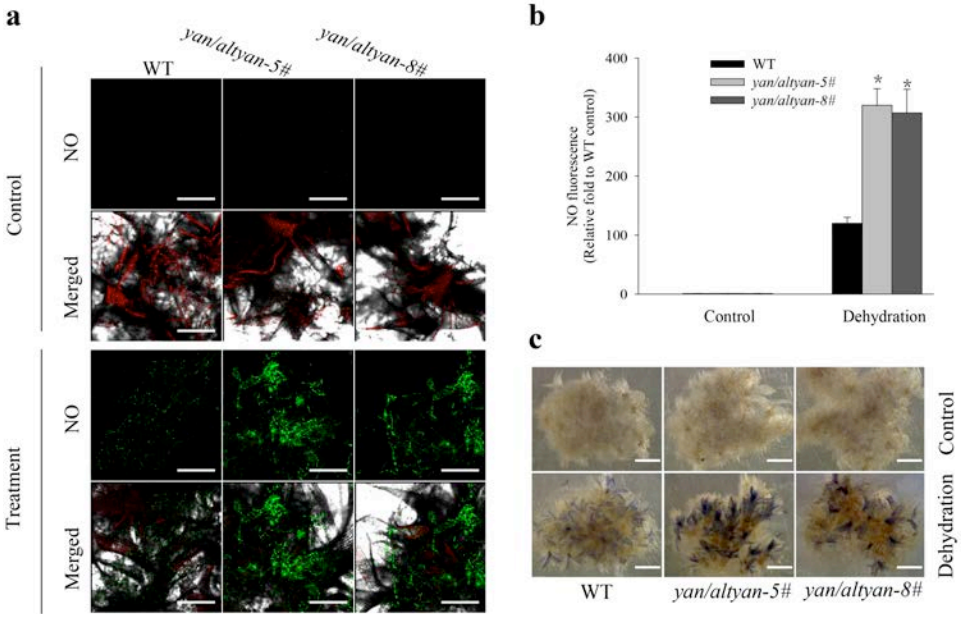
Supplementary Figure 10. Generation of *yan/altyan* knockout mutants and molecular identification. (**a**) Schematic diagram of *YAN/AltYAN* gene disruption construct. The positions of the primers used for PCR analyses of wild-type plants and mutants are indicated with arrows. (**b**) The transformants were tested by PCR on genomic DNA using primer pairs shown in schematic diagram. (**c**) *YAN* and *AltYAN* transcriptions were detected in wild-type plants and *yan/altyan* mutants using semi-quantitative RT-PCR. Primers specific to *ACTIN* were used as the control gene. As shown in the figure, *YAN* and *AltYAN* transcriptions were abolished in *yan/altyan* knockout mutants.



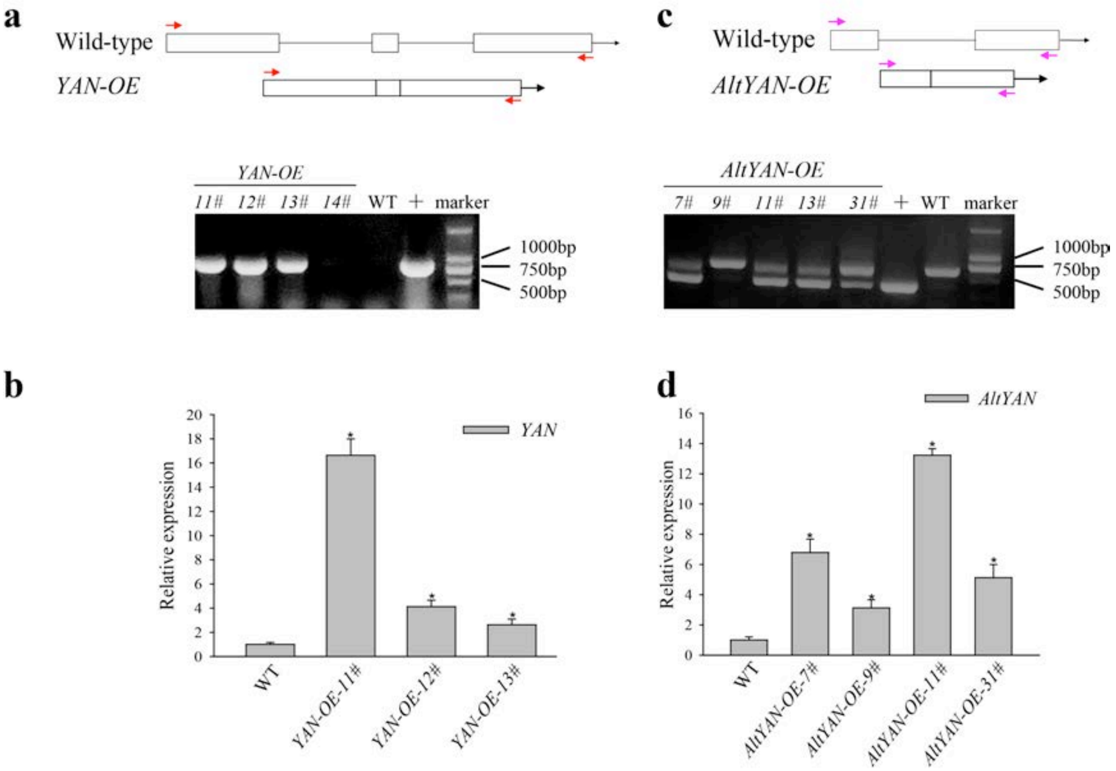
WT *yan/altyan-5#* *yan/altyan-8#* *yan/altyan-13#* *yan/altyan-36#* *yan/altyan-59#*

AltYAN-OE-7# *AltYAN-OE-11#* *AltYAN-OE-13#* *AltYAN-OE-31#* *YAN-OE-11#* *YAN-OE-12#* *YAN-OE-13#*

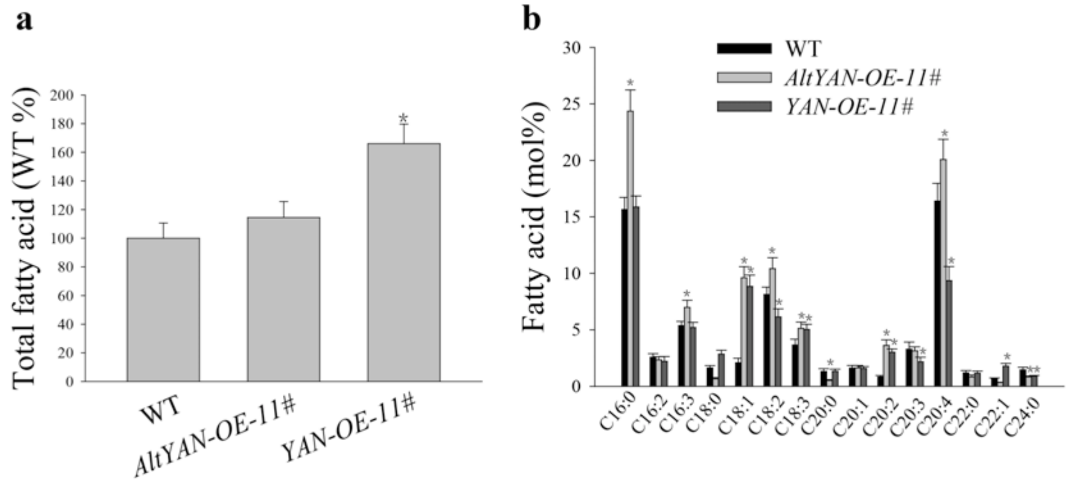
Supplementary Figure 11. Chromosome ploidy analyses. The chromosome ploidy of 2-week old plants was detected using flow cytometry.



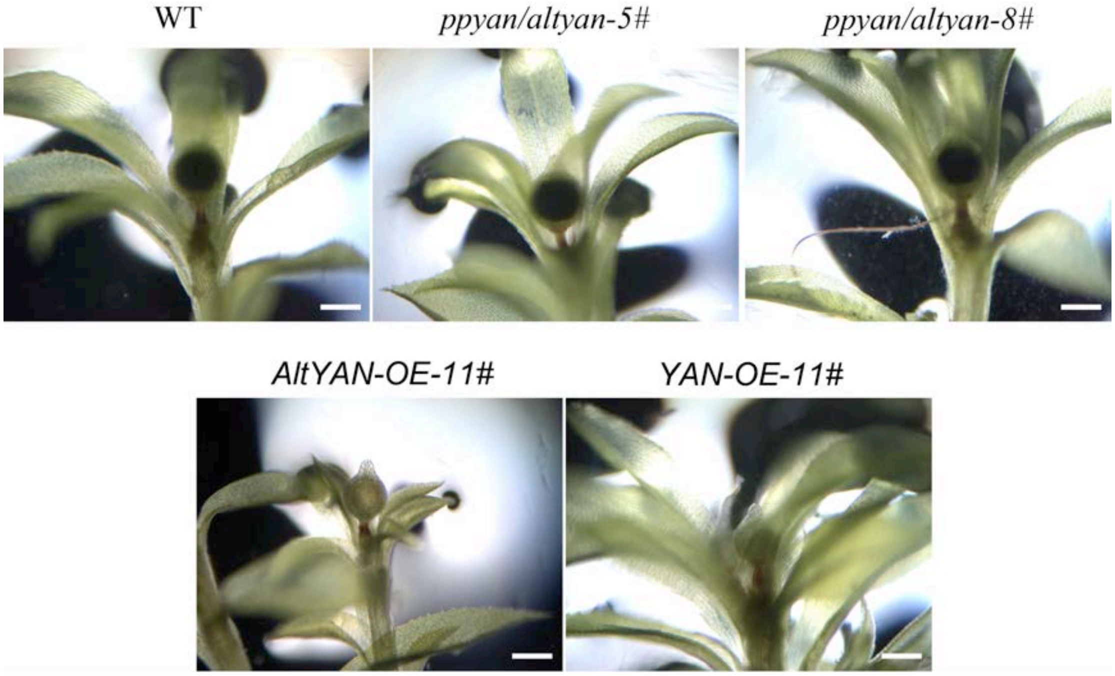
Supplementary Figure 12. Nitric oxide (NO) and superoxide anion accumulation in wild-type plants and *yan/altyan* knockout mutants. Two-week old *P. patens* plants were dehydrated for 20 hours and transferred into sterile water for 1 hour. Plants were then transferred onto standard medium for recovery. Superoxide anions were detected following two days of recovery. (**a**) Toxic NO accumulation in wild-type plants and *yan/altyan.* More toxic NO can be observed in *yan/altyan* mutants than wild-type plants under water stress. NO (green), chlorophyll (red). Scale bar = 1 mm. (**b**) Quantitative analyses of NO fluorescence in *yan/altyan* mutants and wild-type plant using image J software. Values show means and standard deviations of three biological replicates for the wild-type and two independent mutants. Asterisks indicate a statistically significant difference compared with wild-type plants based on a two-tailed student’s *t*-test ($p<0.05$). Scale bar = 2 mm. (**c**) Comparison of superoxide anion accumulation in *yan/altyan* mutants and wild-type plants under dehydration stress. Dark brown areas show superoxide anions.



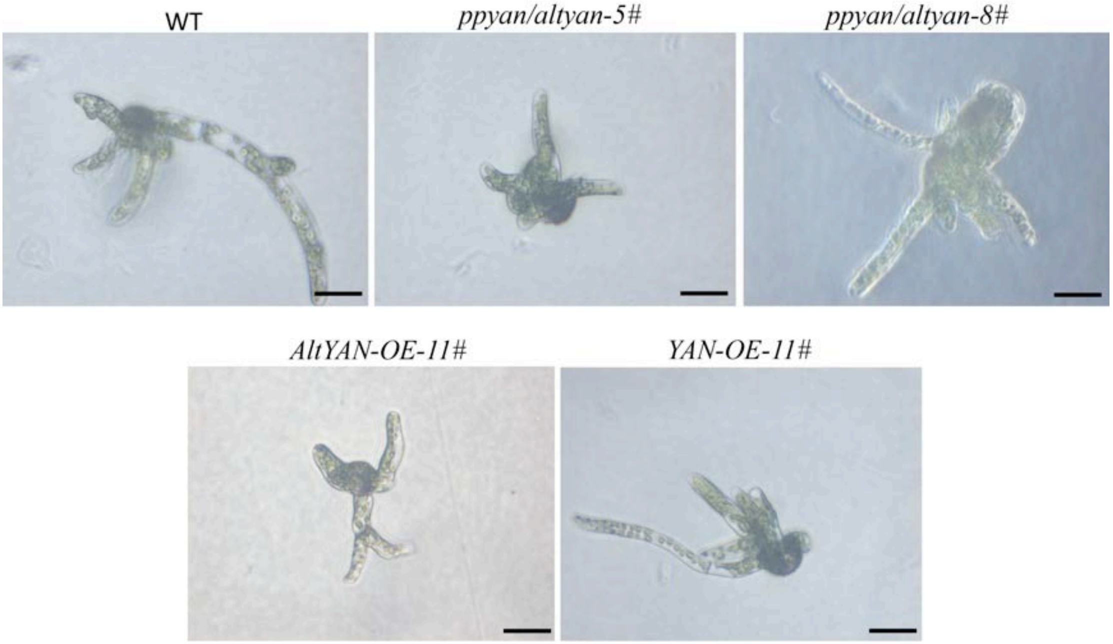
Supplementary Figure 13. Genotyping of *YAN-OE* and *AltYAN-OE* lines. (**a**) Genomic PCR was performed using DNA templates of *YAN-OE,* wild-type (negative control), and *pPOG1-YAN* (positive control). A primer pair designed to amplify full-length CDS of *YAN* was used. (**b**) Quantitative PCR was used to detect the expression level of *YAN* in *YAN-OE* lines. Values show means and standard deviations of three biological replicates for the wild-type and three *YAN-OE* lines. Asterisks indicate a statistically significant difference compared with wild-type plants based on a two-tailed student's *t*-test ($p<0.05$). (**c**) Genomic PCR was performed using DNA templates of *AltYAN-OE,* wild-type (negative control), and *pPOG1-AltYAN* (positive control). A primer pair designed to amplify full-length CDS of *AltYAN* was used. (**d**) Quantitative PCR was used to detect the expression level of *AltYAN* in *AltYAN-OE* lines. Values show means and standard deviations of three biological replicates for the wild-type and four *AltYAN-OE* lines. Asterisks indicate a statistically significant difference compared with wild-type plants based on a two-tailed student's *t*-test ($p<0.05$).



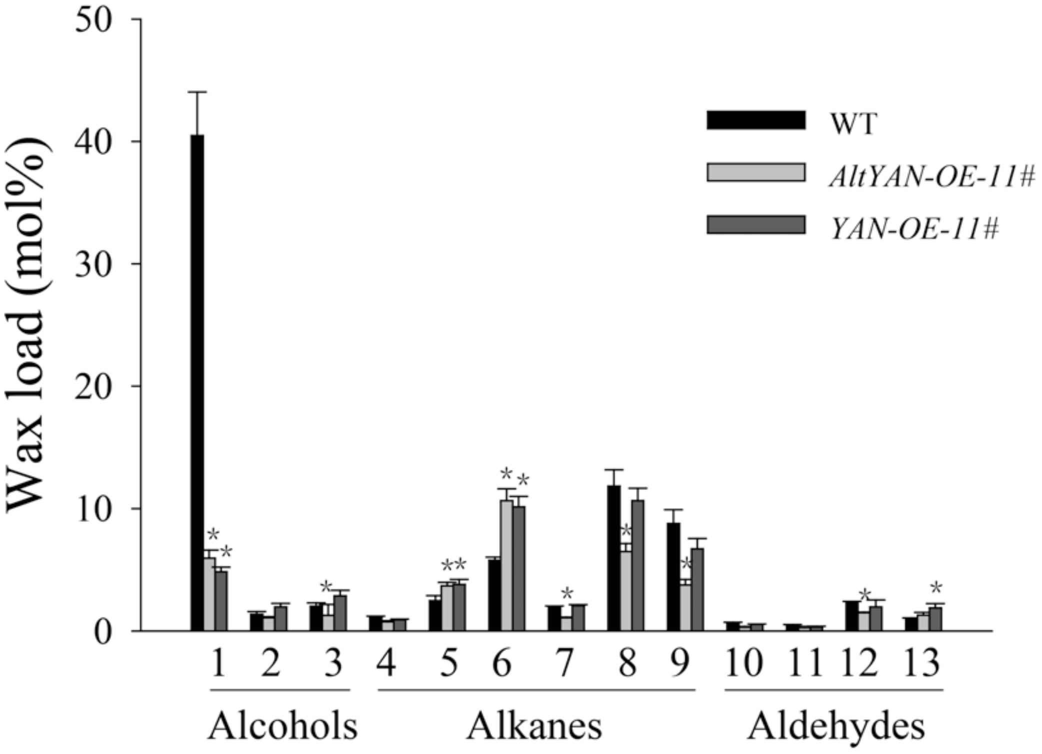
Supplementary Figure 14. Quantification of fatty acids in wild-type, *AltYAN-OE* and *YAN-OE* lines. Ten-week old plants were used to detect the composition and total amount of fatty acids. Values show means and standard deviations of three biological replicates for the wild-type, *AltYAN-OE-11#* and *YAN-OE-11#*. Asterisks indicate a statistically significant difference compared with wild-type plants based on a two-tailed student's *t*-test (p<0.05). (**a**) Total fatty acid content in the wild-type, *AltYAN-OE* and *YAN-OE* lines. **(b**) Comparison of fatty acid amount and composition in wild-type, *AltYAN-OE* and *YAN-OE* lines.



Supplementary Figure 15. Sporophytes of the wild-type and mutants. Plants were grown for 8 weeks at 25 °C before they were transferred to a short-day regime at 15 °C. After 4 weeks of sporophyte induction, visible sporophytes were photographed. Scale bar = 100 µm.



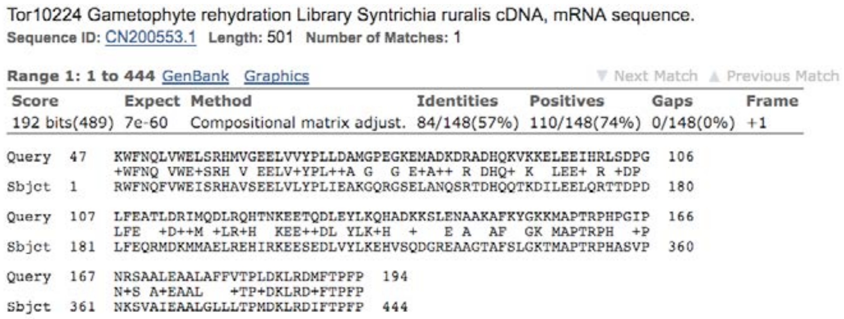
Supplementary Figure 16. Germination of spores from wild-type and mutant plants of *Physcomitrella patens*. Mature sporangia were sterilized using 10% sodium hypocholorite solution for 10 minutes, then washed 5 times using sterilized water. The sporangia were crushed using the tip of a pipet. The released spores were spread on BCDAT medium. The germinated spores were photographed after 4 days of incubation. Scale bar = 50 µm.



1. 16-Hydroxy ent-kaurane 2. 1-Tetracosanol 3. 1-Hexacosanol 4. Octadecane-1-bromo 5. Kaur-15-ene 6. Ent-kaurene 7. 1,19-Eicosadiene 8. 1,21-Docosadiene 9. 1,21-Tetracosene 10. Octadecanal 11. Eicosenel 12. Docosanal 13. Tetracosanal

Supplementary Figure 17. Quantification of wax in wild-type, *AltYAN-OE* and *YAN-OE* lines. Ten-week old plants were used to detect the composition of wax. Values show means and standard deviations of three biological replicates for the wild-type, *AltYAN-OE-11#* and *YAN-OE-11#*. Asterisks indicate a statistically significant difference compared with wild-type plants based on a two-tailed student’s *t*-test (p<0.05).

a



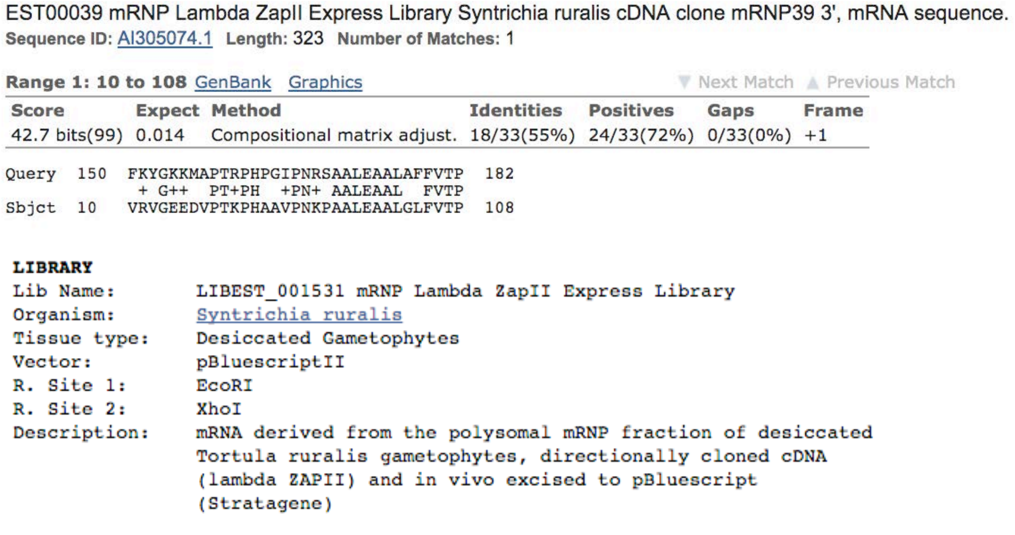
Tor10224 Gametophyte rehydration Library Syntrichia ruralis cDNA, mRNA sequence.
Sequence ID: CN200553.1 **Length:** 501 **Number of Matches:** 1

Range 1: 1 to 444 GenBank Graphics

Score	Expect	Method	Identities	Positives	Gaps	Frame
192 bits(489)	7e-60	Compositional matrix adjust.	84/148(57%)	110/148(74%)	0/148(0%)	+1

```
Query  47   KWFNQLVWELSRHMVGEELVVYPLLDAMGPEGKEMADKDRADHQKVKKELEEIHRLSDPG  106
            +WFNQ VWE+SRH V EELV+YPL++A G  G E+A++ R DHQ+ K  LEE+ R +DP
Sbjct  1    RWFNQFVWEISRHAVSEELVLYPLIEAKGQRGSELANQSRTDHQQTKDILEELQRTTDPD  180

Query  107  LFEATLDRIMQDLRQHTNKEETQDLEYLKQHADKKSLENAAKAFKYGKKMAPTRPHPGIP  166
            LFE  +D++M +LR+H  KEE++DL YLK+H  +   E A  AF  GK MAPTRPH  +P
Sbjct  181  LFEQRMDKMMAELREHIRKEESEDLVYLKEHVSQDGREAAGTAFSLGKTMAPTRPHASVP  360

Query  167  NRSAALEAALAFFVTPLDKLRDMFTPFP  194
            N+S A+EAAL   +TP+DKLRD+FTPFP
Sbjct  361  NKSVAIEAALGLLLTPMDKLRDIFTPFP  444
```

b



EST00039 mRNP Lambda ZapII Express Library Syntrichia ruralis cDNA clone mRNP39 3', mRNA sequence.
Sequence ID: AI305074.1 **Length:** 323 **Number of Matches:** 1

Range 1: 10 to 108 GenBank Graphics

Score	Expect	Method	Identities	Positives	Gaps	Frame
42.7 bits(99)	0.014	Compositional matrix adjust.	18/33(55%)	24/33(72%)	0/33(0%)	+1

```
Query  150  FKYGKKMAPTRPHPGIPNRSAALEAALAFFVTP  182
             + G++  PT+PH  +PN+ AALEAAL  FVTP
Sbjct  10   VRVGEEDVPTKPHAAVPNKPAALEAALGLFVTP  108
```

LIBRARY

Lib Name:	LIBEST_001531 mRNP Lambda ZapII Express Library
Organism:	Syntrichia ruralis
Tissue type:	Desiccated Gametophytes
Vector:	pBluescriptII
R. Site 1:	EcoRI
R. Site 2:	XhoI
Description:	mRNA derived from the polysomal mRNP fraction of desiccated Tortula ruralis gametophytes, directionally cloned cDNA (lambda ZAPII) and in vivo excised to pBluescript (Stratagene)

c

SLA006H09_34729 An expressed sequence tag (EST) collection from the resurrection plant Selaginella lepidophylla Selaginella lepidophylla cDNA clone SLA006H09 5, mRNA sequence.
Sequence ID: BM402674.1 **Length:** 590 **Number of Matches:** 1

Range 1: 249 to 590 GenBank Graphics

Score	Expect	Method	Identities	Positives	Gaps	Frame
125 bits(313)	7e-33	Compositional matrix adjust.	58/114(51%)	82/114(71%)	0/114(0%)	+3

```
Query  14   DLMDEIKHDHREFEEFYTNYKRAYAAGDEKEAEKWFNQLVWELSRHMVGEELVVYPLLDA  73
            D++++I  DH E +++Y  YK A    D++EA KWFNQ VWE+SRH V EELV+YPLL+
Sbjct  249  DIIEKITTDHGELDDYYKKYKSAVKGRDKEEALKWFNQFVWEVSRHSVSEELVLYPLLER  428

Query  74   MGPEGKEMADKDRADHQKVKKELEEIHRLSDPGLFEATLDRIMQDLRQHTNKEE  127
             G +GKE+ADK R+DH KVK+ L E+  ++DP  FE  +D +M++LR+H   EE
Sbjct  429  FGDKGKELADKSRSDHHKVKEYLAEMQSINDPLEFEPKMDEMMKELREHIKMEE  590
```

An expressed sequence tag (EST) collection from the resurrection plant Selaginella lepidophylla

Identifiers	BioSample: SAMN00170085; EST: LIBEST_010176
Organism	Selaginella lepidophylla cellular organisms; Eukaryota; Viridiplantae; Streptophyta; Streptophytina; Embryophyta; Tracheophyta; Lycopodiopsida; Selaginellales; Selaginellaceae; Selaginella
Attributes	**development stage** adult **tissue** microphyll fronds undergoing desiccation for 2.5 h **re_1** EcoRI **v_type** Phagemid **re_2** XhoI **vector** Lambda Uni-Zap XR, Bluescript SK-
Description	Library construction was performed according to manufacture's (Stratagene, Inc.) recommended protocol for the Lambda UniZapXR vector and cDNA synthesis kit.
Submission	University of Nevada, Cushman JC; 2002-01-22

Accession: SAMN00170085 ID: 170085
EST

Supplementary Figure 18. *Hr* genes are induced by dehydration and rehydration in two resurrection plants, the twisted moss *Syntrichia ruralis* and the spikemoss *Selaginella lepidophylla*. The Hr protein sequence from *Sphagnum fallax* (Phytozome identifier Sphfalx0048s0034.1) was used as query to perform TBLASTN search of NCBI dbEST database. **(a)** Alignment of *Sphagnum* Hr protein sequences with a translated sequence of *S. ruralis* rehydration transcript. **(b)** Alignment of *Sphagnum* Hr protein sequences with the translated sequence of another *S. ruralis* transcript, with the associated biosample record indicating that the sample was under desiccation. **(c)** Alignment of *Sphagnum* Hr protein sequences with a translated sequence of *S. lepidophylla* EST, with the associated biosample record indicating that the sample was under desiccation for 2.5 hours.

Supplementary Table 1. TBLASTN result of 1KP database using a *Sphagnum* Hr protein sequence as query; Query=Sphfalx0048s0034.1 peptide (212 amino acids); E-value cutoff = 0.01.

```
--------------------------------------------------------------------------------------------------------
                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value


  scaffold-GOWD-2080086-Sphagnum_lescurii                             285     7e-95
  scaffold-RDOO-2010340-Racomitrium_varium                            227     4e-70
  scaffold-WOGB-2015424-Andreaea_rupestris                            219     4e-68
  scaffold-FFPD-2009361-Ceratodon_purpureus                           221     9e-68
  scaffold-RDOO-2116375-Racomitrium_varium                            218     3e-67
  scaffold-VMXJ-2128859-Leucobryum_albidum                            220     7e-67
  scaffold-KEFD-2005771-Encalypta_streptocarpa                        214     3e-66
  scaffold-ABCD-2074154-Niphotrichum_elongatum                        213     1e-65
  scaffold-RDOO-2116980-Racomitrium_varium                            211     5e-64
  scaffold-HRWG-2013098-Buxbaumia_aphylla                             207     1e-63
  scaffold-BPSG-2019772-Scouleria_aquatica                            205     3e-62
  scaffold-WOGB-2092690-Andreaea_rupestris                            204     8e-62
  scaffold-LGDQ-2041893-Selaginella_apoda                             199     2e-61
  scaffold-TXVB-2071911-Lunularia_cruciata                            199     4e-60
  scaffold-GRKU-2004786-Syntrichia_princeps                           193     3e-59
  scaffold-JKAA-2008718-Selaginella_wallacei                          199     3e-59
  scaffold-ABCD-2010707-Niphotrichum_elongatum                        196     4e-59
  scaffold-JKAA-2008719-Selaginella_wallacei                          199     6e-59
  scaffold-NGTD-2098183-Dicranum_scoparium                            196     1e-58
  scaffold-JMXW-2003398-Bryum_argenteum                               187     1e-56
  scaffold-ZYCD-2004286-Selaginella_acanthonota                       196     1e-56
  scaffold-JMXW-2003397-Bryum_argenteum                               187     3e-56
  scaffold-JMXW-2003396-Bryum_argenteum                               186     6e-56
  scaffold-ZZOL-2052205-Selaginella_stauntoniana                      185     5e-55
  scaffold-ZYCD-2004285-Selaginella_acanthonota                       196     8e-55
  scaffold-ORKS-2011502-Philonotis_fontana                            191     5e-53
  scaffold-GRKU-2012644-Syntrichia_princeps                           179     7e-53
  scaffold-PKOX-2012929-Isoetes_tegetiformans                         181     1e-52
  scaffold-GRKU-2012643-Syntrichia_princeps                           180     1e-52
  scaffold-VMXJ-2124089-Leucobryum_albidum                            173     3e-51
  scaffold-RDOO-2109788-Racomitrium_varium                            169     9e-51
  scaffold-FITN-2089742-Treubia_lacunose                              177     9e-51
  scaffold-PYHZ-2006808-Isoetes_sp                                    177     2e-50
  scaffold-PKOX-2096679-Isoetes_tegetiformans                         175     4e-50
```

```
scaffold-TMAJ-2173065-Neckera_douglasii                            161     2e-47
scaffold-YPIC-2134561-Microstegium_vimineum                        149     5e-42
scaffold-RXRQ-2128249-Phaeoceros_carolinianus-sporophyte           145     5e-41
scaffold-WCZB-2013633-Phaeoceros_carolinianus-gametophyte          146     5e-41
scaffold-JMXW-2040177-Bryum_argenteum                              146     6e-41
scaffold-WNGH-2006956-Aulacomnium_heterostichum                    145     3e-40
scaffold-OFTV-2128708-Barbilophozia_barbata                        144     1e-39
scaffold-CMEQ-2082481-Orthotrichum_lyellii                         144     2e-39
scaffold-JBGU-2011382-Amaranthus_palmeri                           143     2e-39
scaffold-YEPO-2071721-cf._Physcomicromitrium_sp                    140     6e-39
scaffold-WCZB-2114770-Phaeoceros_carolinianus-gametophyte          142     4e-38
scaffold-RXRQ-2022078-Phaeoceros_carolinianus-sporophyte           144     4e-38
scaffold-IRBN-2152420-Scapania_nemorosa                            139     1e-37
scaffold-RGKI-2054671-Leucobryum_glaucum                           134     4e-37
scaffold-JKAA-2174841-Selaginella_wallacei                         137     4e-37
scaffold-RXRQ-2132940-Phaeoceros_carolinianus-sporophyte           136     3e-36
scaffold-FFPD-2008086-Ceratodon_purpureus                          131     4e-36
scaffold-FAJB-2053787-Paraphymatoceros_hallii                      137     8e-36
scaffold-RXRQ-2018681-Phaeoceros_carolinianus-sporophyte           136     2e-35
scaffold-GRKU-2004785-Syntrichia_princeps                          132     4e-35
scaffold-YBQN-2122714-Odontoschisma_prostratum                     130     9e-35
scaffold-JHFI-2105756-Pellia_neesiana                              129     2e-34
scaffold-CMEQ-2081739-Orthotrichum_lyellii                         129     5e-34
scaffold-TGKW-2009714-Frullania                                    126     3e-33
scaffold-LNSF-2063071-Hypnum_subimponens                           125     4e-33
scaffold-KJZG-2059461-Asplenium_platyneuron                        129     5e-33
scaffold-LNSF-2008214-Hypnum_subimponens                           127     7e-33
scaffold-IRBN-2150099-Scapania_nemorosa                            125     8e-33
scaffold-NOKI-2092089-Lindsaea_linearis                            128     1e-32
scaffold-ABCD-2011017-Niphotrichum_elongatum                       129     1e-32
scaffold-NWQC-2149828-Plagiochila_asplenioides                     126     2e-32
scaffold-IRBN-2150967-Scapania_nemorosa                            125     2e-32
scaffold-JKAA-2177860-Selaginella_wallacei                         127     3e-32
scaffold-GSXD-2062693-Myriopteris_eatonii                          124     5e-32
scaffold-OFTV-2123041-Barbilophozia_barbata                        121     9e-32
scaffold-MZLD-2123763-Ligustrum_sinense                            124     1e-31
scaffold-DCDT-2072974-Cheilanthes_arizonica                        125     1e-31
scaffold-KJZG-2058634-Asplenium_platyneuron                        124     2e-31
scaffold-HNDZ-2129005-Cystopteris_utahensis                        122     3e-31
scaffold-JBGU-2121651-Amaranthus_palmeri                           123     3e-31
scaffold-LNSF-2008215-Hypnum_subimponens                           122     4e-31
```

```
scaffold-JKAA-2170971-Selaginella_wallacei                          119     8e-31
scaffold-OCZL-2010080-Homalosorus_pycnocarpos                       122     1e-30
scaffold-ABCD-2009871-Niphotrichum_elongatum                        122     1e-30
scaffold-IGUH-2161180-Schwetschkeopsis_fabronia                     120     1e-30
scaffold-WNGH-2085370-Aulacomnium_heterostichum                     121     3e-30
scaffold-NWQC-2005566-Plagiochila_asplenioides                      120     4e-30
scaffold-NWQC-2005567-Plagiochila_asplenioides                      118     9e-30
scaffold-CQPW-2020236-Anemia_tomentosa                              119     3e-29
scaffold-CPKP-2097340-Lophophora_williamsii                         114     1e-28
scaffold-WFBF-2000168-Podophyllum_peltatum                          114     2e-28
scaffold-JKAA-2167480-Selaginella_wallacei                          111     5e-28
scaffold-NRWZ-2091968-Metzgeria_crassipilis                         110     1e-27
scaffold-XDDT-2012577-Argyrochosma_nivea                            112     2e-27
scaffold-IRBN-2142735-Scapania_nemorosa                             110     2e-27
scaffold-RDOO-2109148-Racomitrium_varium                            106     4e-26
scaffold-WOGB-2081836-Andreaea_rupestris                            103     1e-25
scaffold-OSHQ-2044949-Sarcandra_glabra                              106     2e-25
scaffold-ZTHV-2000232-Atrichum_angustatum                           103     2e-24
scaffold-NRWZ-2009763-Metzgeria_crassipilis                         99.4    2e-23
scaffold-WTDE-2023700-Johnsonia_pubescens                           99.8    2e-23
scaffold-IGUH-2154130-Schwetschkeopsis_fabronia                     99.4    2e-23
scaffold-SALZ-2065380-Pittosporum_resiniferum-fruit                 100     8e-23
scaffold-WCZB-2102726-Phaeoceros_carolinianus-gametophyte           96.3    2e-22
scaffold-CHJJ-2127518-Lejeuneaceae_sp                               96.7    2e-22
scaffold-CIEA-2049265-Juncus_inflexus                               98.6    2e-22
scaffold-NGTD-2090279-Dicranum_scoparium                            96.3    3e-22
scaffold-IRBN-2139892-Scapania_nemorosa                             95.9    5e-22
scaffold-VQYB-2010950-Neurachne_lanigera                            95.5    2e-21
scaffold-ABCD-2064447-Niphotrichum_elongatum                        93.6    2e-21
scaffold-OCWZ-2051463-Dioscorea_villosa                             97.1    5e-21
scaffold-PSHB-2008862-Lantana_camara                                94.0    7e-21
scaffold-KEFD-2050257-Encalypta_streptocarpa                        91.7    9e-21
scaffold-KEFD-2054814-Encalypta_streptocarpa                        92.8    1e-20
scaffold-VMXJ-2107202-Leucobryum_albidum                            89.0    5e-20
scaffold-YWNF-2008347-Hedwigia_ciliata                              90.9    1e-19
scaffold-RGKI-2054107-Leucobryum_glaucum                            88.2    2e-19
scaffold-TMAJ-2001356-Neckera_douglasii                             92.0    2e-19
scaffold-YWNF-2008346-Hedwigia_ciliata                              91.7    4e-19
scaffold-RTMU-2149348-Calypogeia_fissa                              86.7    7e-19
scaffold-HATH-2079510-Aristida_stricta                              87.0    9e-19
scaffold-ZXJO-2012394-Hemionitis_arifolia                           87.0    3e-18
```

scaffold-YPIC-2132251-Microstegium_vimineum	86.3	4e-18
scaffold-WVMY-2126909-Phlox_drummondii	86.3	4e-18
scaffold-ABIJ-2031254-Selaginella_lepidophylla	83.2	9e-18
scaffold-MVSE-2025297-Griselinia_littoralis	85.5	9e-18
scaffold-RDOO-2015219-Racomitrium_varium	85.5	1e-17
scaffold-IRBN-2122784-Scapania_nemorosa	80.9	7e-17
scaffold-VMXJ-2115109-Leucobryum_albidum	81.6	7e-17
scaffold-UEEN-2146356-Forestiera_segregata	80.5	1e-16
scaffold-YPIC-2127709-Microstegium_vimineum	81.3	1e-16
scaffold-NWQC-2113770-Plagiochila_asplenioides	80.1	1e-16
scaffold-IRBN-2155004-Scapania_nemorosa	85.1	2e-16
scaffold-WNGH-2012483-Aulacomnium_heterostichum	83.2	2e-16
scaffold-YPIC-2115414-Microstegium_vimineum	79.0	3e-16
scaffold-POPJ-2014900-Pteris_vittata	80.5	4e-16
scaffold-PKOX-2008033-Isoetes_tegetiformans	59.7	9e-16
scaffold-IGUH-2105402-Schwetschkeopsis_fabronia	77.0	1e-15
scaffold-QMWB-2052150-Anomodon_attenuatus	78.6	1e-15
scaffold-NGTD-2089101-Dicranum_scoparium	79.0	1e-15
scaffold-BGXB-2069679-Plagiomnium_insigne	78.6	1e-15
scaffold-OCWZ-2048666-Dioscorea_villosa	79.3	2e-15
scaffold-IRBN-2002456-Scapania_nemorosa	78.6	2e-15
scaffold-TVSH-2057737-Bituminaria_bituminosa	77.0	3e-15
scaffold-XWHK-2033525-Funaria	75.9	3e-15
scaffold-NGTD-2072576-Dicranum_scoparium	75.9	3e-15
scaffold-NDUV-2084566-Vittaria_appalachiana	75.9	6e-15
scaffold-TMAJ-2176774-Neckera_douglasii	78.6	1e-14
scaffold-WTDE-2120279-Johnsonia_pubescens	75.5	1e-14
scaffold-WPUV-2050890-Oenothera_grandis-MTJ_135	74.7	3e-14
scaffold-ZENX-2075599-Neurachne_alopecuroidea	73.6	4e-14
scaffold-VMXJ-2101389-Leucobryum_albidum	72.8	6e-14
scaffold-XWDM-2024866-XWDM-Cystopteris_fragilis-2_samples_combined	62.0	9e-14
scaffold-CPKP-2083294-Lophophora_williamsii	72.0	2e-13
scaffold-CVEG-2117220-Azolla_cf._caroliniana	72.4	2e-13
scaffold-RTMU-2146607-Calypogeia_fissa	72.0	3e-13
scaffold-PYHZ-2058919-Isoetes_sp	65.9	3e-13
scaffold-TMAJ-2146025-Neckera_douglasii	70.1	8e-13
scaffold-UPMJ-2080149-Pseudolycopodiella_caroliniana	73.6	1e-12
scaffold-ZTHV-2078155-Atrichum_angustatum	59.3	1e-12
scaffold-RTMU-2082020-Calypogeia_fissa	68.6	1e-12
scaffold-CMEQ-2067654-Orthotrichum_lyellii	68.9	2e-12
scaffold-RTMU-2159248-Calypogeia_fissa	70.9	2e-12

```
scaffold-KEFD-2052864-Encalypta_streptocarpa                        70.5    2e-12
scaffold-CMEQ-2063716-Orthotrichum_lyellii                          67.4    4e-12
scaffold-YPIC-2024478-Microstegium_vimineum                         70.1    5e-12
scaffold-HATH-2073870-Aristida_stricta                              68.2    6e-12
scaffold-PMRP-2036296-Cannabis_sativa-stem                          70.1    1e-11
scaffold-BJSW-2066960-BJSW-Cannabis_sativa-4_samples_combined       70.1    1e-11
scaffold-IMZV-2080047-IMZV-Oenothera_grandis-5_samples_combined     67.0    2e-11
scaffold-RXRQ-2131384-Phaeoceros_carolinianus-sporophyte            69.7    2e-11
scaffold-OCWZ-2044044-Dioscorea_villosa                             66.2    3e-11
scaffold-CHJJ-2130531-Lejeuneaceae_sp                               68.2    3e-11
scaffold-GKAG-2079692-Huperzia_lucidula                             65.1    5e-11
scaffold-IGUH-2160559-Schwetschkeopsis_fabronia                     68.6    6e-11
scaffold-CHJJ-2012733-Lejeuneaceae_sp                               68.6    6e-11
scaffold-KRUQ-2055797-Porella_navicularis                           63.2    1e-10
scaffold-VMXJ-2081240-Leucobryum_albidum                            63.5    1e-10
scaffold-JKAA-2154702-Selaginella_wallacei                          64.3    1e-10
scaffold-JSAG-2054274-Masdevallia_yuangensis                        62.8    2e-10
scaffold-FITN-2084753-Treubia_lacunose                              65.9    2e-10
scaffold-GRKU-2069920-Syntrichia_princeps                           63.5    3e-10
scaffold-ABCD-2067800-Niphotrichum_elongatum                        64.3    5e-10
scaffold-IGUH-2157080-Schwetschkeopsis_fabronia                     64.7    6e-10
scaffold-RTMU-2014569-Calypogeia_fissa                              46.6    6e-10
scaffold-NRWZ-2079339-Metzgeria_crassipilis                         62.4    8e-10
scaffold-NRWZ-2092900-Metzgeria_crassipilis                         64.3    1e-09
scaffold-LHLE-2056411-Cystopteris_fragilis                          61.6    1e-09
scaffold-HATH-2079179-Aristida_stricta                              63.2    1e-09
scaffold-UUHD-2077091-Porella_pinnata                               60.5    1e-09
scaffold-LNSF-2055857-Hypnum_subimponens                            62.0    2e-09
scaffold-FFPD-2029090-Ceratodon_purpureus                           59.7    2e-09
scaffold-TMAJ-2012211-Neckera_douglasii                             62.8    2e-09
scaffold-MIRS-2073569-Climacium_dendroides                          61.2    3e-09
scaffold-HPXA-2104167-Ptilidium_pulcherrimum                        60.5    3e-09
scaffold-CHJJ-2000052-Lejeuneaceae_sp                               63.2    4e-09
scaffold-GRKU-2071783-Syntrichia_princeps                           60.8    5e-09
scaffold-VMXJ-2123744-Leucobryum_albidum                            63.2    5e-09
scaffold-HVBQ-2112238-Tetraphis_pellucida                           60.5    6e-09
scaffold-QMWB-2055650-Anomodon_attenuatus                           62.0    6e-09
scaffold-HPXA-2127478-Ptilidium_pulcherrimum                        62.0    7e-09
scaffold-WOGB-2084446-Andreaea_rupestris                            60.5    7e-09
scaffold-JKAA-2078739-Selaginella_wallacei                          57.8    1e-08
scaffold-AEXY-2130519-Blasia_sp                                     60.8    1e-08
```

```
scaffold-JKAA-2165817-Selaginella_wallacei                            60.5    1e-08
scaffold-PSHB-2043956-Lantana_camara                                  58.2    1e-08
scaffold-NGTD-2089411-Dicranum_scoparium                              60.1    1e-08
scaffold-JMXW-2034222-Bryum_argenteum                                 58.5    2e-08
scaffold-TMAJ-2003433-Neckera_douglasii                               58.9    2e-08
scaffold-NRWZ-2085378-Metzgeria_crassipilis                           58.9    2e-08
scaffold-KEFD-2044179-Encalypta_streptocarpa                          57.4    4e-08
scaffold-GRRW-2048048-Grevillea_robusta                               57.8    4e-08
scaffold-ABCD-2058843-Niphotrichum_elongatum                          57.4    5e-08
scaffold-PSHB-2039572-Lantana_camara                                  55.5    9e-08
scaffold-XNXF-2001698-Dendrolycopodium_obscurum                       53.5    1e-07
scaffold-IRBN-2103858-Scapania_nemorosa                               55.5    2e-07
scaffold-TMWO-2076697-Papaver_bracteatum-bulb                         54.7    2e-07
scaffold-SSDU-2123337-SSDU-Papaver_bracteatum-3_samples_combined      54.7    2e-07
scaffold-VMXJ-2120453-Leucobryum_albidum                              57.4    2e-07
scaffold-JHCN-2084608-Oxalis_sp                                       57.0    3e-07
scaffold-NRWZ-2088588-Metzgeria_crassipilis                           56.2    3e-07
scaffold-UAXP-2066247-Gyrostemon_ramulosus                            55.5    3e-07
scaffold-IGUH-2005101-Schwetschkeopsis_fabronia                       57.4    3e-07
scaffold-NRWZ-2089194-Metzgeria_crassipilis                           56.2    4e-07
scaffold-RTMU-2146256-Calypogeia_fissa                                55.5    4e-07
scaffold-VMXJ-2077554-Leucobryum_albidum                              53.9    5e-07
scaffold-OFTV-2003852-Barbilophozia_barbata                           57.4    5e-07
scaffold-ZXJO-2005224-Hemionitis_arifolia                             55.8    6e-07
scaffold-ABIJ-2034612-Selaginella_lepidophylla                        55.5    8e-07
scaffold-YEPO-2030533-cf._Physcomicromitrium_sp                       52.8    8e-07
scaffold-WOGB-2008726-Andreaea_rupestris                              55.8    9e-07
scaffold-CHJJ-2116743-Lejeuneaceae_sp                                 53.9    1e-06
scaffold-IRBN-2005941-Scapania_nemorosa                               56.2    2e-06
scaffold-IRBN-2005942-Scapania_nemorosa                               56.2    2e-06
scaffold-IRBN-2076572-Scapania_nemorosa                               52.0    2e-06
scaffold-ABCD-2014511-Niphotrichum_elongatum                          51.2    3e-06
scaffold-OFTV-2087677-Barbilophozia_barbata                           42.0    3e-06
scaffold-HPXA-2008638-Ptilidium_pulcherrimum                          38.9    3e-06
scaffold-RDOO-2089861-Racomitrium_varium                              52.0    4e-06
scaffold-IRBN-2155117-Scapania_nemorosa                               55.1    5e-06
scaffold-IGUH-2005100-Schwetschkeopsis_fabronia                       53.9    5e-06
scaffold-YPIC-2124849-Microstegium_vimineum                           52.4    6e-06
scaffold-HPXA-2105951-Ptilidium_pulcherrimum                          51.6    7e-06
scaffold-MROH-2036845-Thelypteris_acuminata                           52.0    7e-06
scaffold-ZSAB-2180975-Hoheria_angustifolia                            52.0    9e-06
```

```
scaffold-YCKE-2055091-Notholaena_montieliae                         52.8    9e-06
scaffold-TMAJ-2120109-Neckera_douglasii                             50.8    9e-06
scaffold-LRTN-2087772-Monotropa_uniflora                            51.6    1e-05
scaffold-GRKU-2044556-Syntrichia_princeps                           49.7    1e-05
scaffold-CHJJ-2119525-Lejeuneaceae_sp                               51.2    2e-05
scaffold-NGTD-2066643-Dicranum_scoparium                            49.7    2e-05
scaffold-CMEQ-2082860-Orthotrichum_lyellii                          53.1    3e-05
scaffold-WOGB-2051835-Andreaea_rupestris                            48.5    3e-05
scaffold-MVSE-2190933-Griselinia_littoralis                         49.3    4e-05
scaffold-PSHB-2043518-Lantana_camara                                43.1    5e-05
scaffold-HATH-2002294-Aristida_stricta                              49.7    5e-05
scaffold-JKAA-2053839-Selaginella_wallacei                          48.1    5e-05
scaffold-YWNF-2020517-Hedwigia_ciliata                              47.8    5e-05
scaffold-YWNF-2020516-Hedwigia_ciliata                              47.8    5e-05
scaffold-FROP-2070730-FROP-Epifagus_virginiana-2_samples_combined   49.3    6e-05
scaffold-WCZB-2085267-Phaeoceros_carolinianus-gametophyte           48.5    6e-05
scaffold-BJSW-2012126-BJSW-Cannabis_sativa-4_samples_combined       50.1    7e-05
scaffold-WFBF-2003972-Podophyllum_peltatum                          51.2    7e-05
scaffold-WOGB-2008722-Andreaea_rupestris                            49.3    8e-05
scaffold-VMXJ-2036090-Leucobryum_albidum                            47.0    9e-05
scaffold-OQWW-2161174-Davallia_fejeensis                            47.4    1e-04
scaffold-YPIC-2134240-Microstegium_vimineum                         50.1    2e-04
scaffold-KRUQ-2090857-Porella_navicularis                           47.8    2e-04
scaffold-NGTD-2062919-Dicranum_scoparium                            47.0    2e-04
scaffold-OXYP-2078939-Sideroxylon_reclinatum                        46.6    2e-04
scaffold-KEFD-2052641-Encalypta_streptocarpa                        48.5    2e-04
scaffold-XRCX-2095148-Aster_tataricus                               48.5    2e-04
scaffold-CHJJ-2127168-Lejeuneaceae_sp                               48.5    3e-04
scaffold-JKAA-2061455-Selaginella_wallacei                          45.8    3e-04
scaffold-CVEG-2094112-Azolla_cf._caroliniana                        46.2    4e-04
scaffold-OQWW-2161093-Davallia_fejeensis                            45.8    4e-04
scaffold-JKAA-2008453-Selaginella_wallacei                          47.8    4e-04
scaffold-LNSF-2045802-Hypnum_subimponens                            46.2    5e-04
scaffold-NRWZ-2086705-Metzgeria_crassipilis                         47.4    5e-04
scaffold-FAJB-2050601-Paraphymatoceros_hallii                       47.8    6e-04
scaffold-NDUV-2080635-Vittaria_appalachiana                         45.8    7e-04
scaffold-VMXJ-2028561-Leucobryum_albidum                            44.7    7e-04
scaffold-TGKW-2021476-Frullania                                     44.3    0.001
scaffold-EWXK-2077938-Thyrsopteris_elegans                          44.7    0.001
scaffold-RDOO-2025299-Racomitrium_varium                            43.9    0.001
scaffold-KEFD-2043942-Encalypta_streptocarpa                        45.1    0.001
```

scaffold-VMXJ-2028149-Leucobryum_albidum	43.5	0.002
scaffold-VBMM-2023838-Anomodon_rostratus	43.5	0.002
scaffold-JKAA-2129507-Selaginella_wallacei	43.9	0.002
scaffold-PMRP-2006102-Cannabis_sativa-stem	45.4	0.003
scaffold-NRWZ-2069162-Metzgeria_crassipilis	43.9	0.003
scaffold-NDUV-2096264-Vittaria_appalachiana	45.8	0.004
scaffold-WOGB-2064377-Andreaea_rupestris	43.1	0.005
scaffold-NRWZ-2047801-Metzgeria_crassipilis	42.0	0.009
scaffold-CHJJ-2131097-Lejeuneaceae_sp	44.7	0.009

Supplementary Table 2. Taxonomic report of TBLASTN search of *nr* database using a translated charophyte sequence (ISGT-2038756) as query. The sequence was retrieved from 1KP and translated into six frames. The match to Hr protein sequences was used as query to search the nr databases. E-value cutoff = 1.

Organism	Blast Name	Score	Number of Hits	Description
cellular organisms			167	
.Bacteria	bacteria		159	
..Proteobacteria	proteobacteria		143	
...Alphaproteobacteria	a-proteobacteria		99	
....Rhodospirillales	a-proteobacteria		11	
.....Rhodospirillaceae	a-proteobacteria		4	
......Rhodospirillaceae bacterium CCH5-H10	a-proteobacteria	50.4	1	Rhodospirillaceae bacterium CCH5-H10 hits
......Phaeospirillum fulvum	a-proteobacteria	46.6	1	Phaeospirillum fulvum hits
......Phaeospirillum fulvum MGU-K5	a-proteobacteria	39.7	1	Phaeospirillum fulvum MGU-K5 hits
......Dongia sp. URHE0060	a-proteobacteria	37.4	1	Dongia sp. URHE0060 hits
.....Rhodospirillales bacterium URHD0017	a-proteobacteria	47.8	4	Rhodospirillales bacterium URHD0017 hits
.....Reyranella massiliensis	a-proteobacteria	40.4	1	Reyranella massiliensis hits
.....Rhodospirillales bacterium SCN 65-16	a-proteobacteria	40.0	1	Rhodospirillales bacterium SCN 65-16 hits
.....Enhydrobacter aerosaccus	a-proteobacteria	37.0	1	Enhydrobacter aerosaccus hits
....Sphingobium cloacae	a-proteobacteria	47.0	2	Sphingobium cloacae hits
....Sphingobium baderi	a-proteobacteria	46.6	2	Sphingobium baderi hits
....Sphingobium chungbukense	a-proteobacteria	46.2	2	Sphingobium chungbukense hits
....Sphingobium japonicum	a-proteobacteria	46.2	2	Sphingobium japonicum hits
....Sphingobium japonicum UT26S	a-proteobacteria	46.2	1	Sphingobium japonicum UT26S hits
....Sphingobium	a-proteobacteria	46.2	2	Sphingobium hits
....Sphingobium chinhatense IP26	a-proteobacteria	46.2	1	Sphingobium chinhatense IP26 hits
....Sphingobium sp. HDIP04	a-proteobacteria	46.2	1	Sphingobium sp. HDIP04 hits
....Sphingobium lucknowense F2	a-proteobacteria	46.2	1	Sphingobium lucknowense F2 hits
....Sphingomonadaceae	a-proteobacteria	46.2	2	Sphingomonadaceae hits
....Sphingomonas sp. BHC-A	a-proteobacteria	46.2	1	Sphingomonas sp. BHC-A hits
....Sphingobium indicum B90A	a-proteobacteria	46.2	1	Sphingobium indicum B90A hits
....Sphingobium yanoikuyae	a-proteobacteria	44.7	4	Sphingobium yanoikuyae hits
....Sphingobium sp. YBL2	a-proteobacteria	44.7	1	Sphingobium sp. YBL2 hits
....Sphingobium sp. AM	a-proteobacteria	44.7	1	Sphingobium sp. AM hits
....Sphingobium sp. 22B	a-proteobacteria	44.7	1	Sphingobium sp. 22B hits
....Sphingomonas paucimobilis	a-proteobacteria	44.7	3	Sphingomonas paucimobilis hits
....Sphingomonas sp. MEA3-1	a-proteobacteria	44.7	1	Sphingomonas sp. MEA3-1 hits
....alpha proteobacterium LLX12A	a-proteobacteria	43.1	1	alpha proteobacterium LLX12A hits
....Novosphingobium sp. Rr 2-17	a-proteobacteria	43.1	2	Novosphingobium sp. Rr 2-17 hits
....Caulobacter sp. K31	a-proteobacteria	43.1	2	Caulobacter sp. K31 hits
....Sphingobium sp. DC-2	a-proteobacteria	42.4	1	Sphingobium sp. DC-2 hits
....Caulobacter sp. Root343	a-proteobacteria	42.4	2	Caulobacter sp. Root343 hits
....Caulobacter henricii	a-proteobacteria	42.4	1	Caulobacter henricii hits
....Brevundimonas viscosa	a-proteobacteria	42.4	2	Brevundimonas viscosa hits
....Sphingomonas koreensis	a-proteobacteria	42.0	3	Sphingomonas koreensis hits
....Sphingobium quisquiliarum	a-proteobacteria	42.0	1	Sphingobium quisquiliarum hits
....Sphingobium quisquiliarum P25	a-proteobacteria	42.0	1	Sphingobium quisquiliarum P25 hits
....Sphingobium sp. MI1205	a-proteobacteria	41.2	2	Sphingobium sp. MI1205 hits
....Sphingomonas sp. Root241	a-proteobacteria	41.2	2	Sphingomonas sp. Root241 hits
....Novosphingobium panipatense	a-proteobacteria	41.2	1	Novosphingobium panipatense hits
....Sphingobium herbicidovorans	a-proteobacteria	41.2	1	Sphingobium herbicidovorans hits
....Sphingobium herbicidovorans NBRC 16415	a-proteobacteria	41.2	1	Sphingobium herbicidovorans NBRC 16415 hits
....Sphingomonas pruni	a-proteobacteria	40.8	1	Sphingomonas pruni hits
....Sphingobium sp. YL23	a-proteobacteria	40.0	1	Sphingobium sp. YL23 hits
....Sphingomonas sp. Ndbn-20	a-proteobacteria	40.0	1	Sphingomonas sp. Ndbn-20 hits
....Sphingomonas sp. URHD0057	a-proteobacteria	40.0	1	Sphingomonas sp. URHD0057 hits
....Sphingobium sp. SYK-6	a-proteobacteria	40.0	2	Sphingobium sp. SYK-6 hits
....Sphingomonas	a-proteobacteria	39.7	1	Sphingomonas hits
....Sphingomonas sp. Root1294	a-proteobacteria	39.7	1	Sphingomonas sp. Root1294 hits
....Sphingomonas sp. Root50	a-proteobacteria	39.7	1	Sphingomonas sp. Root50 hits
....Sphingomonas sp. Root720	a-proteobacteria	39.7	1	Sphingomonas sp. Root720 hits
....Sphingomonas sp. MCT13	a-proteobacteria	39.7	2	Sphingomonas sp. MCT13 hits

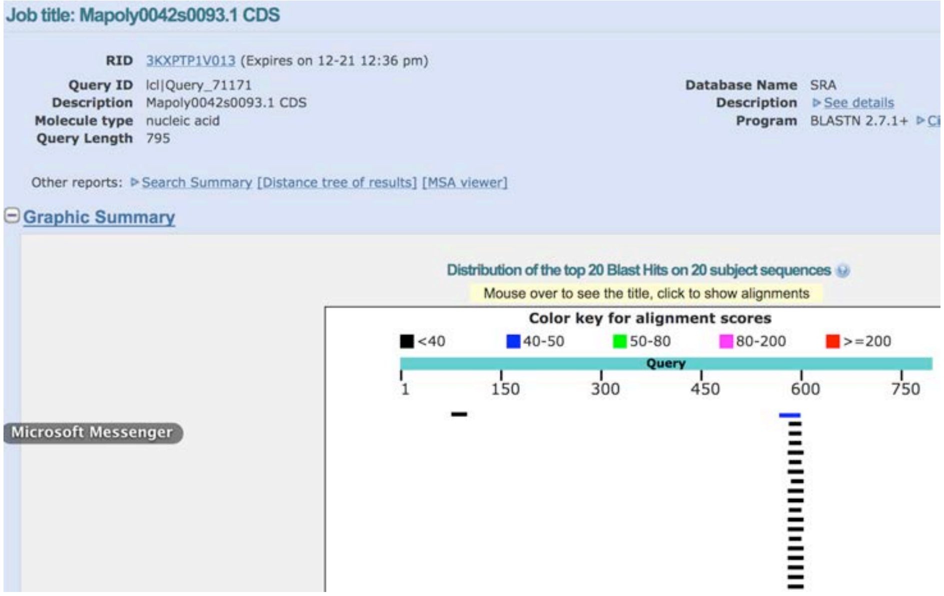
....Sphingomonas sp. JJ-A5	a-proteobacteria	39.3	2	Sphingomonas sp. JJ-A5 hits
....Sphingobium sp. EP60837	a-proteobacteria	39.3	2	Sphingobium sp. EP60837 hits
....Methylocystis sp. SC2	a-proteobacteria	39.3	1	Methylocystis sp. SC2 hits
....Sphingobium sp. Ant17	a-proteobacteria	38.5	2	Sphingobium sp. Ant17 hits
....Caulobacterales bacterium 32-69-10	a-proteobacteria	38.5	1	Caulobacterales bacterium 32-69-10 hits
....Sphingomonas sp. 28-66-16	a-proteobacteria	38.5	1	Sphingomonas sp. 28-66-16 hits
....Sphingomonas sp. UNC305MFCol5.2	a-proteobacteria	38.1	1	Sphingomonas sp. UNC305MFCol5.2 hits
....Novosphingobium sp. MD-1	a-proteobacteria	38.1	1	Novosphingobium sp. MD-1 hits
....Sphingobium sp. YR657	a-proteobacteria	38.1	2	Sphingobium sp. YR657 hits
....Sphingobium yanoikuyae ATCC 51230	a-proteobacteria	38.1	1	Sphingobium yanoikuyae ATCC 51230 hits
....Sphingobium japonicum BiD32	a-proteobacteria	37.4	1	Sphingobium japonicum BiD32 hits
....Brevundimonas	a-proteobacteria	37.0	1	Brevundimonas hits
....Brevundimonas sp. Root1423	a-proteobacteria	37.0	1	Brevundimonas sp. Root1423 hits
....Brevundimonas sp. Root608	a-proteobacteria	37.0	1	Brevundimonas sp. Root608 hits
....Sphingomonas hengshuiensis	a-proteobacteria	37.0	2	Sphingomonas hengshuiensis hits
....Sphingomonas asaccharolytica	a-proteobacteria	37.0	1	Sphingomonas asaccharolytica hits
....Sphingomonas mali	a-proteobacteria	37.0	1	Sphingomonas mali hits
....Sphingobium sp. AP49	a-proteobacteria	36.2	2	Sphingobium sp. AP49 hits
...Burkholderia sp. A27	b-proteobacteria	49.7	2	Burkholderia sp. A27 hits
...Paraburkholderia	b-proteobacteria	47.4	1	Paraburkholderia hits
...Paraburkholderia sediminicola	b-proteobacteria	47.4	1	Paraburkholderia sediminicola hits
...Paraburkholderia terricola	b-proteobacteria	47.4	1	Paraburkholderia terricola hits
...Paraburkholderia terrae	b-proteobacteria	46.2	8	Paraburkholderia terrae hits
...Paraburkholderia terrae BS001	b-proteobacteria	46.6	2	Paraburkholderia terrae BS001 hits
...Burkholderia sp. yr281	b-proteobacteria	46.6	2	Burkholderia sp. yr281 hits
...Paraburkholderia monticola	b-proteobacteria	43.1	2	Paraburkholderia monticola hits
...Mitsuaria sp. 7	b-proteobacteria	42.0	2	Mitsuaria sp. 7 hits
...Burkholderia sp. YR277	b-proteobacteria	41.6	2	Burkholderia sp. YR277 hits
...Aeromonas salmonicida	g-proteobacteria	40.8	4	Aeromonas salmonicida hits
...Nitrosomonas sp. Nm166	b-proteobacteria	40.8	2	Nitrosomonas sp. Nm166 hits
...Burkholderia sp. UYPR1.413	b-proteobacteria	40.8	1	Burkholderia sp. UYPR1.413 hits
...Rhizobacter sp. Root404	b-proteobacteria	40.4	2	Rhizobacter sp. Root404 hits
...Roseateles depolymerans	b-proteobacteria	39.7	2	Roseateles depolymerans hits
...Ramlibacter sp. Leaf400	b-proteobacteria	39.3	2	Ramlibacter sp. Leaf400 hits
...Rhizobacter sp. OV335	b-proteobacteria	38.5	2	Rhizobacter sp. OV335 hits
...Mitsuaria sp. HZ7	b-proteobacteria	37.7	2	Mitsuaria sp. HZ7 hits
...Cellvibrio sp. BR	g-proteobacteria	37.0	2	Cellvibrio sp. BR hits
...Roseateles terrae	b-proteobacteria	36.6	2	Roseateles terrae hits
..Streptomyces sp. F-3	high GC Gram+	40.4	2	Streptomyces sp. F-3 hits
..Actinomadura rubrobrunea	high GC Gram+	40.0	1	Actinomadura rubrobrunea hits
..Phormidium ambiguum	cyanobacteria	39.7	1	Phormidium ambiguum hits
..Phormidium ambiguum IAM M-71	cyanobacteria	39.7	1	Phormidium ambiguum IAM M-71 hits
..Streptomyces sp. TP-A0356	high GC Gram+	39.7	1	Streptomyces sp. TP-A0356 hits
..Streptomyces sp. e14	high GC Gram+	39.3	1	Streptomyces sp. e14 hits
..Oscillatoria sp. PCC 10802	cyanobacteria	38.1	1	Oscillatoria sp. PCC 10802 hits
..Chloroflexi bacterium 54-19	GNS bacteria	38.5	1	Chloroflexi bacterium 54-19 hits
..Nocardia africana	high GC Gram+	37.7	1	Nocardia africana hits
..Cyanothece sp. PCC 7822	cyanobacteria	37.4	2	Cyanothece sp. PCC 7822 hits
..Micromonospora echinaurantiaca	high GC Gram+	36.2	2	Micromonospora echinaurantiaca hits
..Streptomyces alni	high GC Gram+	36.2	2	Streptomyces alni hits
.Gonium pectorale	green algae	47.4	1	Gonium pectorale hits
.Monoraphidium neglectum	green algae	46.2	2	Monoraphidium neglectum hits
.Phlebia centrifuga	basidiomycetes	41.2	1	Phlebia centrifuga hits
.Sphaerobolus stellatus SS14	basidiomycetes	40.4	1	Sphaerobolus stellatus SS14 hits
.Rhizophagus irregularis DAOM 181602	glomeromycetes	38.5	1	Rhizophagus irregularis DAOM 181602 hits
.Rhizophagus irregularis DAOM 197198w	glomeromycetes	38.5	1	Rhizophagus irregularis DAOM 197198w hits
.Gonapodya prolifera JEL478	monoblepharidomycetes	37.7	1	Gonapodya prolifera JEL478 hits

Supplementary Note 1. With Hr protein sequences from various nonvascular plants and green algae as query, the only two hits from seed plants in the NCBI nucleotide sequence database belong to *Quercus suber* (Fagaceae). These two hits were located on two separate scaffolds of the unpublished draft genome sequence of this species, and they share 88% protein sequence identity. Both sequences are significantly more similar to fungal homologs than to other green plant sequences (up to 68% protein sequence identities with fungi versus 36-37% with the seedless vascular plant *Selaginella moellendorffii*). It is unclear whether these two sequences are derived from sequencing contamination or horizontal gene transfer.

The many hemerythrin hits from flowering plants raises a significant concern about contamination in 1KP data. Other than chlorophycean sequences, which can be verified through the scaffold information of their complete genomes, other algal sequences are only sporadically found in various lineages, including red algae (*Glaucosphera vacuolata*, 1KP IDs: RSOF_scaffold_2008604, RSOF_scaffold_2041944, RSOF_scaffold_2004035, RSOF_scaffold_2032925), glaucophytes (*Glaucocystis nostochinearum*, 1KP IDs: POOW_scaffold_2004542, POOW_scaffold_2053347) and charophytes (*Hormidiella sp.*, 1KP IDs: ISGT-scaffold_2038756, ISGT-scaffold_2001291; *Coleochaete irregulares*, 1KP IDs: QPDY-scoffold_2002876, QPDY-scaffold_2002877), and other chlorophytes (*Chlorella* IKP ID: MWAN_scaffold_2004366; *Parachlorella kessleri,* IKP ID: AKCR_scaffold_2002030; *Ignatius tetrasporus,* 1KP ID: KADG_scaffold_2037975; *Botryococcus_sudeticus,* IKP ID: VJDZ_scaffold_2007822). Because the 1KP database only contains plant sequence data, we further searched the *nr* database using the above sequences as query to investigate whether they were indeed similar to chlorophycean and land plant hemerythrins. These analyses showed that the red algal sequences were significantly more similar to fungal homologs than to chlorophycean and land plant sequences. Not hits to red algae were identified, even though multiple red algal genomes (e.g., *Cyanodioschyzon merolae, Chondrus crispus, Galdieria sulphuraria, Porphyridium purpureum, Porphyra umbilicalis,* and *Pyropia yezoensis*) have been sequenced and deposited in NCBI. Similarly, the glaucophytes were significantly more similar to fungal and bacterial sequences than to chlorophycean and land plant sequences (in both sequence coverage and identity).

Of the four charophyte sequences, three (1KP IDs: ISGT-scaffold_2038756, QPDY-scaffold_2002876 and QPDY-scaffold_2002877) shared higher or similar percent identities with bacterial and fungal homologs (see **Supplementary Table 2** above), and one (1KP ID: ISGT-scaffold_2001291) was slightly more similar to chlorophycean sequences. Although many other green algae outside Chlorophyceae (e.g., the charophyte *Klebsormidium nitens,* chlorophytes *Ostreococcus tauri, Ostreococcus lucimarinus, Micromonas pusilla, Cymbomonas tetramitiformis, Coccomyxa subellipsoidea, Chlorella variabilis, Chlorella vulgaris, Auxenochlorella protothecoides, Picochlorum sp., Helicosporidium sp.*) have been sequenced and their data have been deposited in NCBI, no hits from these taxa were detected. Except for *Parachlorella kessleri*, all other chlorophyte sequences also shared a higher sequence coverage and identity with bacterial or fungal homologs, and again no hits from green algae outside Chlorophyceae were detected. The *Parachlorella kessleri* sequence was more similar to a protein sequence of *Chlorella variabilis* that was translated from mRNA data (NCBI accession number: XP_005847154), which in turn was more similar to fungal and bacterial sequences.

Supplementary Note 2. We searched the *Nitella hyalina* transcriptomic data at NCBI (https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR064326), but only two matches of 19-32 nucleotides were found; these two matches were mapped onto different regions (separated by about 470 nucleotides) of the *Hr* CDS (see figure below, top panel). Search of the transcriptomic data of the charophyte [*Closterium peracerosum-strigosum-littorale complex*]() at NCBI (https://www.ncbi.nlm.nih.gov/bioproject?term=PRJNA296352) provided a similar result (also see below, lower panel).



Job title: Mapoly0042s0093.1 CDS

RID 3KXPTP1V013 (Expires on 12-21 12:36 pm)
Query ID lcl|Query_71171
Description Mapoly0042s0093.1 CDS
Molecule type nucleic acid
Query Length 795

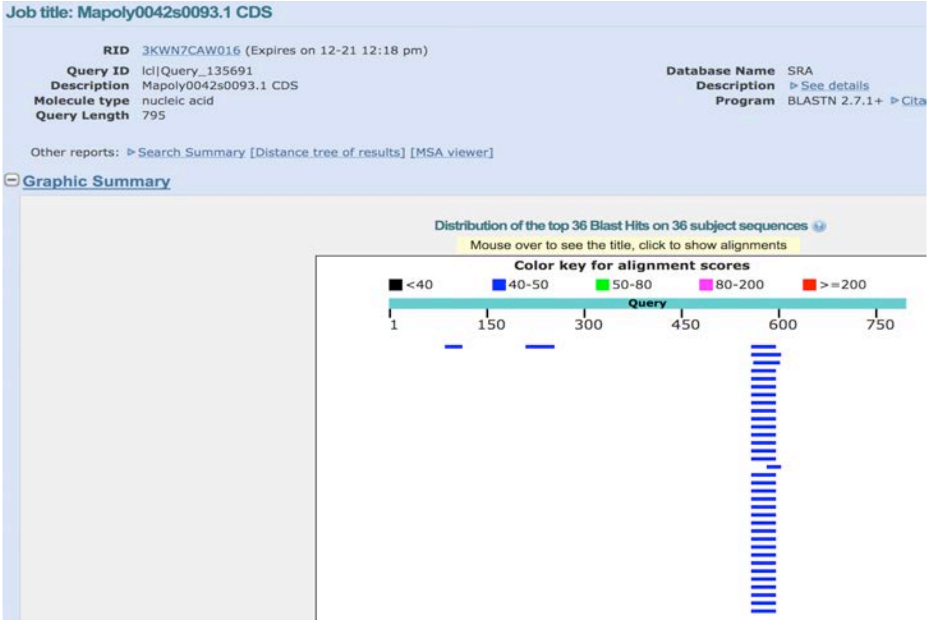
Database Name SRA
Description ▷ See details
Program BLASTN 2.7.1+ ▷ Ci

Other reports: ▷ Search Summary [Distance tree of results] [MSA viewer]

Graphic Summary

Distribution of the top 20 Blast Hits on 20 subject sequences

Mouse over to see the title, click to show alignments



Job title: Mapoly0042s0093.1 CDS

RID 3KWN7CAW016 (Expires on 12-21 12:18 pm)
Query ID lcl|Query_135691
Description Mapoly0042s0093.1 CDS
Molecule type nucleic acid
Query Length 795

Database Name SRA
Description ▷ See details
Program BLASTN 2.7.1+ ▷ Cita

Other reports: ▷ Search Summary [Distance tree of results] [MSA viewer]

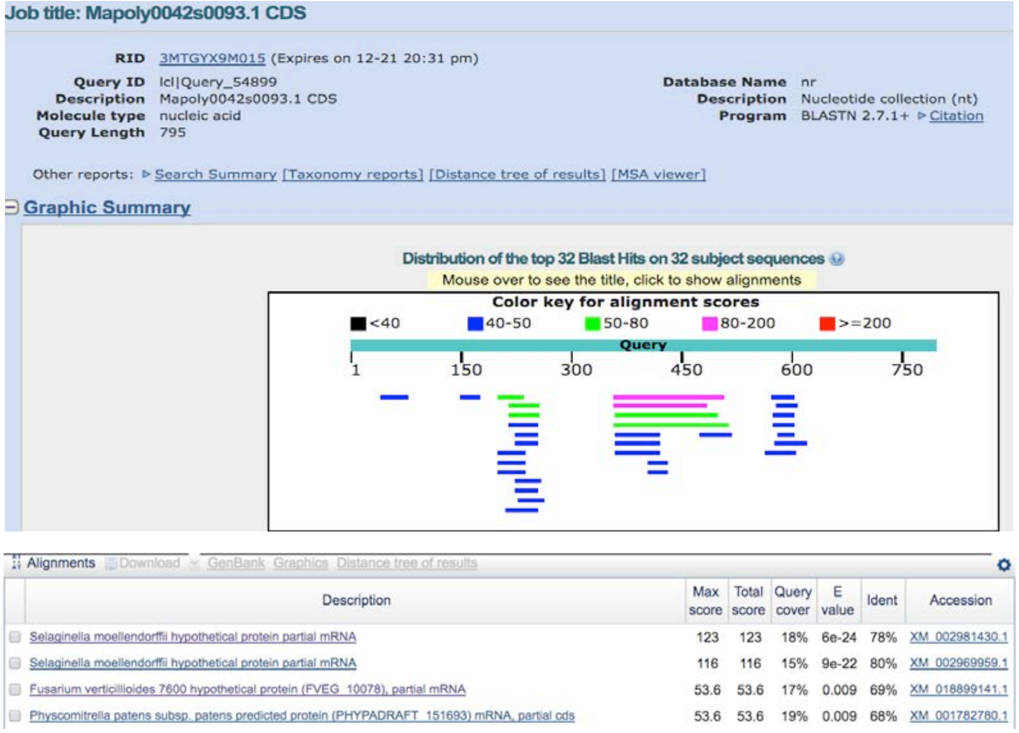
Graphic Summary

Distribution of the top 36 Blast Hits on 36 subject sequences

Mouse over to see the title, click to show alignments

BLASTN search of transcriptomic data of charophytes *Nitella hyalina* (top panel) and *Closterium peracerosum-strigosum-littorale* complex (lower panel) at NCBI. Query is the CDS of *Marchantia polymorpha Hr* (JGI Id: Mapoly0042s0093). Results indicate that only short fragments are mapped to the query.

Because the assessment of sequence homology is more difficult at the level of nucleotide sequences, lack of sufficient coverage to the query in the above search does not necessarily suggest the absence of *Hr* in the two charophytes. Therefore, we further investigated whether the 2-3 charophyte hits were specific to *Hr* (i.e., the HHE domain). To this end, we performed the BLASTN search of NCBI non-redundant nucleotide sequence database (nt) using the same *Marchantia polymorpha Hr* (JGI Id: Mapoly0042s0093) as query. Indeed, the results included hits corresponding to those from the two charophytes (*Nitella hyalina* and *Closterium peracerosum-strigosum-littorale* complex) (see figure below, top panel). However, further inspections of these hits indicated that they were not particular to the *Hr* gene. For instance, the hits to the 670-600 bp region of the query were annotated as part of the genes encoding small integral membrane protein 10-like protein 2A, peroxidase 7-like protein, and myomegalin-like protein; they were found in both flowering plants (*Lupinus angustifolius*, *Vitis venifera*, and *Cucurbita maxima*) and animals (*Crocodylus porosus*, Oncorhynchus mykiss, and *Columba livia*). Similarly, hits to the 150-200 bp region of the query were annotated as genes encoding erythrocyte membrane protein, RP1 like 1 (rp1l1) protein, and retrotransposon Gag like 5 (Rtl5) protein; they could be found in animals (e.g., *Oryzias latipes*, *Heterocephalus glaber*, *Labrus bergylta*) and apicomplexan parasites (*Plasmodium falciparum*). On the other hand, the most significant hits, which also had the longest query coverages, were from *Selaginella moellendorffii*, the fungus *Fusarium verticillioides* and *Physcomitrella patens* (see figure below, lower panel). The former two were part of the *Hr* gene, whereas the later (i.e. *P. patens*) evolved directly from *Hr*. These data suggest that the hits from the two charophytes (*Nitella hyalina* and *Closterium peracerosum-strigosum-littorale* complex) might be associated with genes other than *Hr*.



Job title: Mapoly0042s0093.1 CDS

RID 3MTGYX9M015 (Expires on 12-21 20:31 pm)

Query ID lcl|Query_54899
Description Mapoly0042s0093.1 CDS
Molecule type nucleic acid
Query Length 795

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.7.1+ ▷ Citation

Other reports: ▷ Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

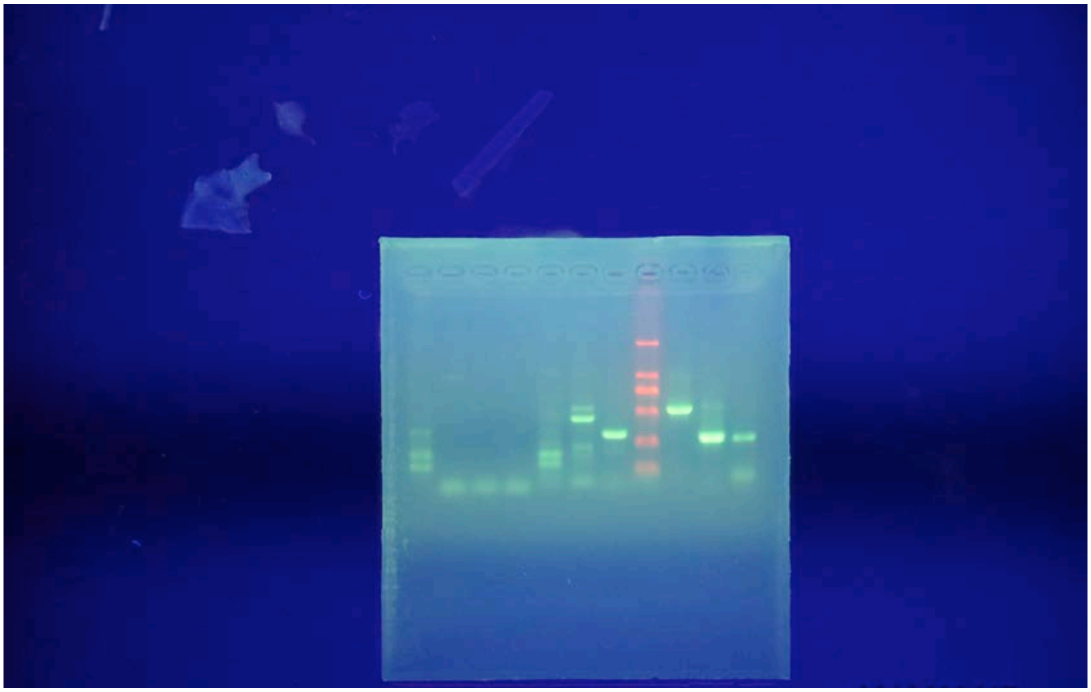
⊟ Graphic Summary

Distribution of the top 32 Blast Hits on 32 subject sequences

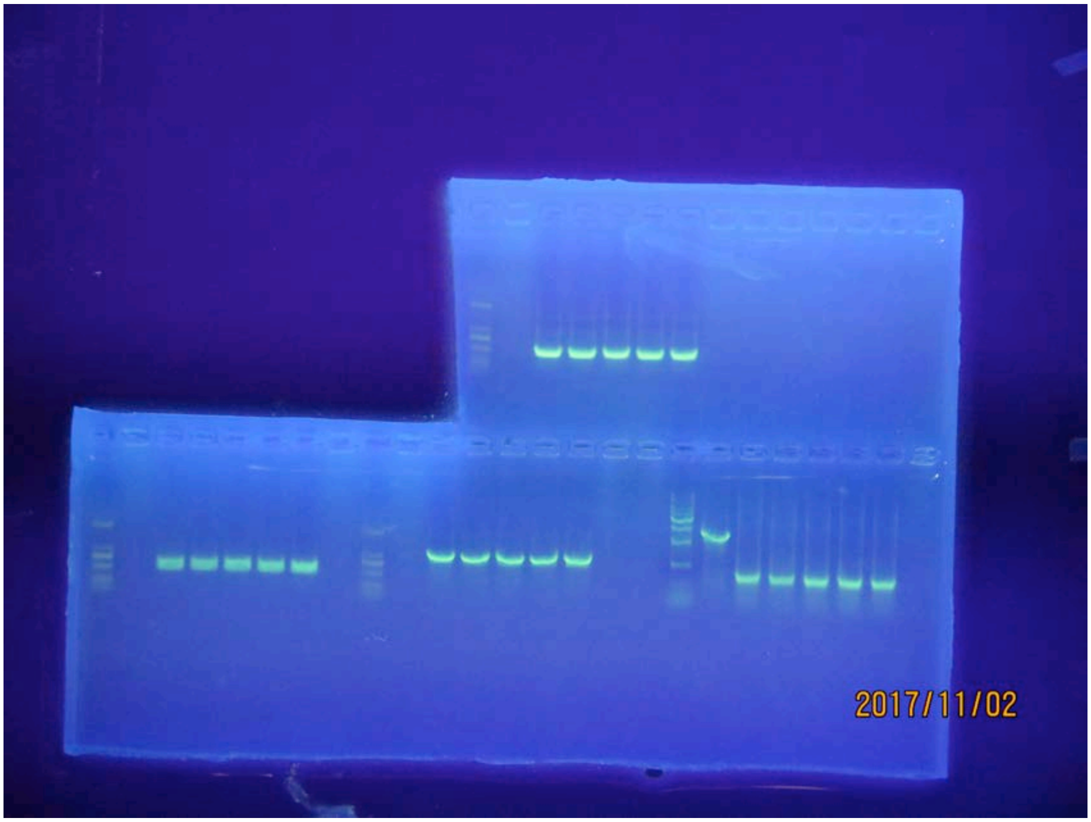
Mouse over to see the title, click to show alignments

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Selaginella moellendorffii hypothetical protein partial mRNA	123	123	18%	6e-24	78%	XM_002981430.1
☐	Selaginella moellendorffii hypothetical protein partial mRNA	116	116	15%	9e-22	80%	XM_002969959.1
☐	Fusarium verticillioides 7600 hypothetical protein (FVEG_10078), partial mRNA	53.6	53.6	17%	0.009	69%	XM_018899141.1
☐	Physcomitrella patens subsp. patens predicted protein (PHYPADRAFT_151693) mRNA, partial cds	53.6	53.6	19%	0.009	68%	XM_001782780.1

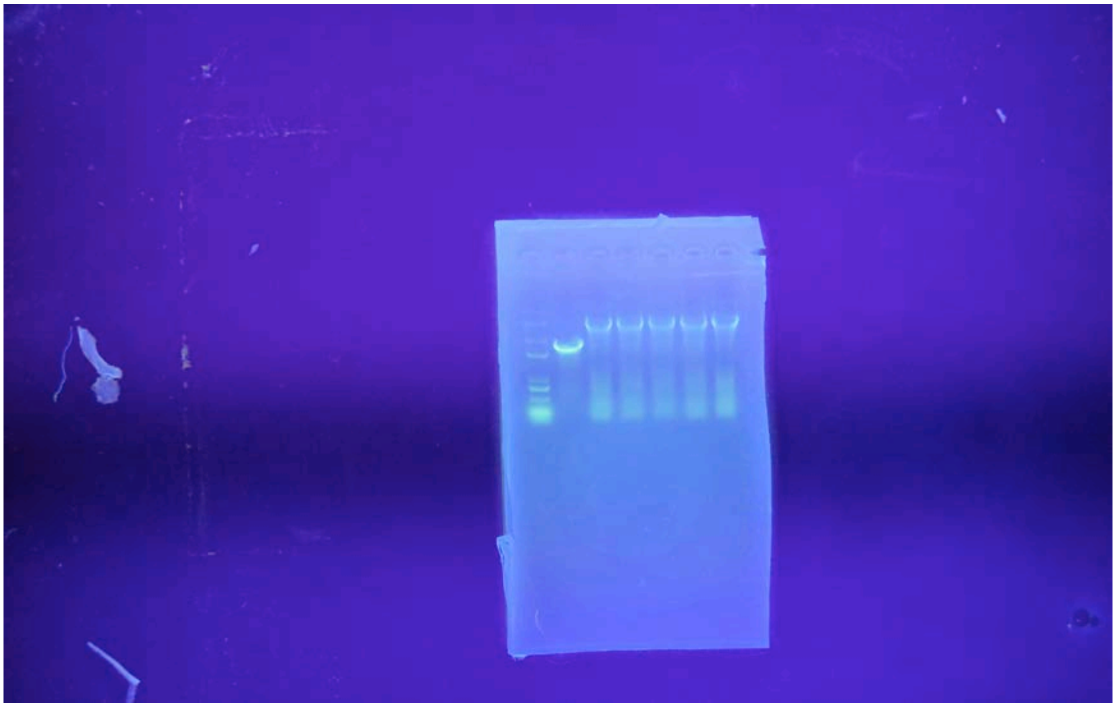
The following includes uncropped gel images for corresponding Supplementary Figures above.



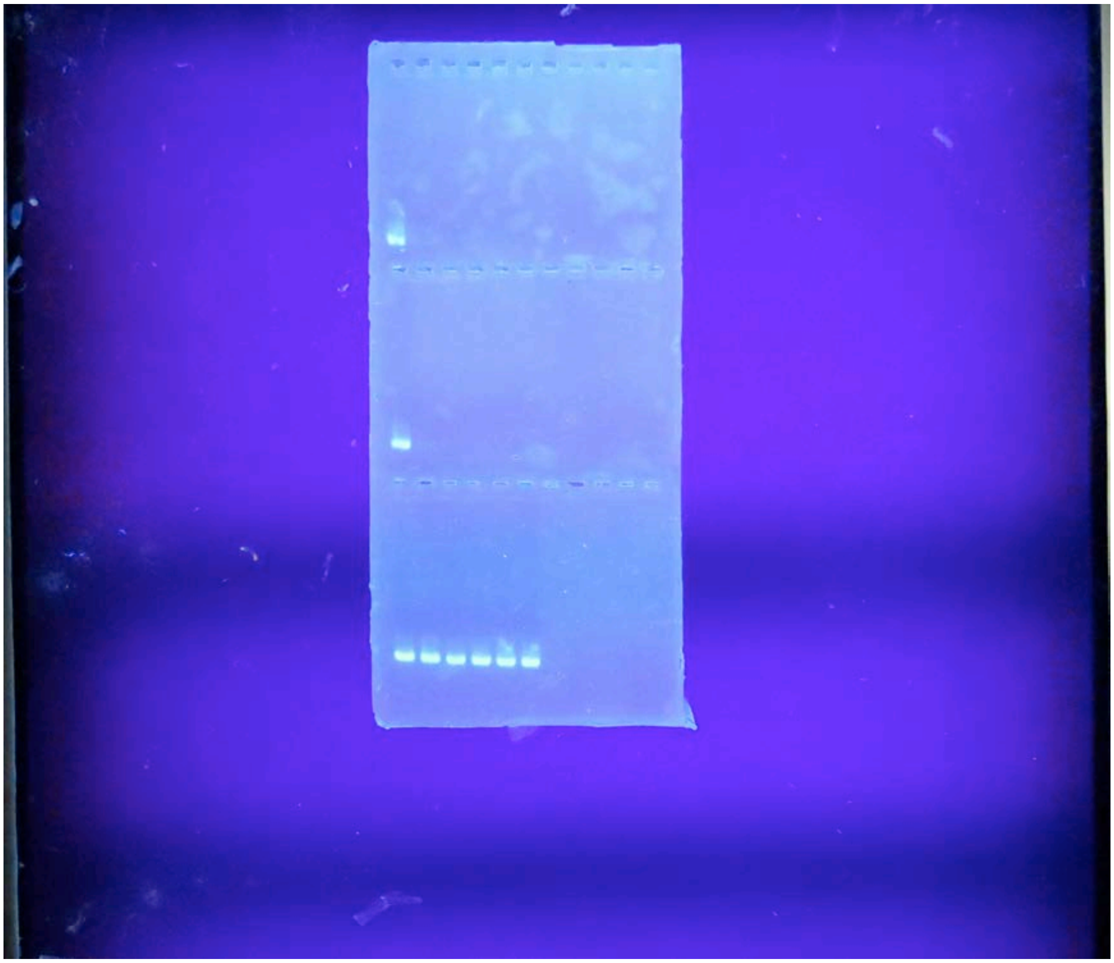
Uncropped gel of supplementary figure 6b



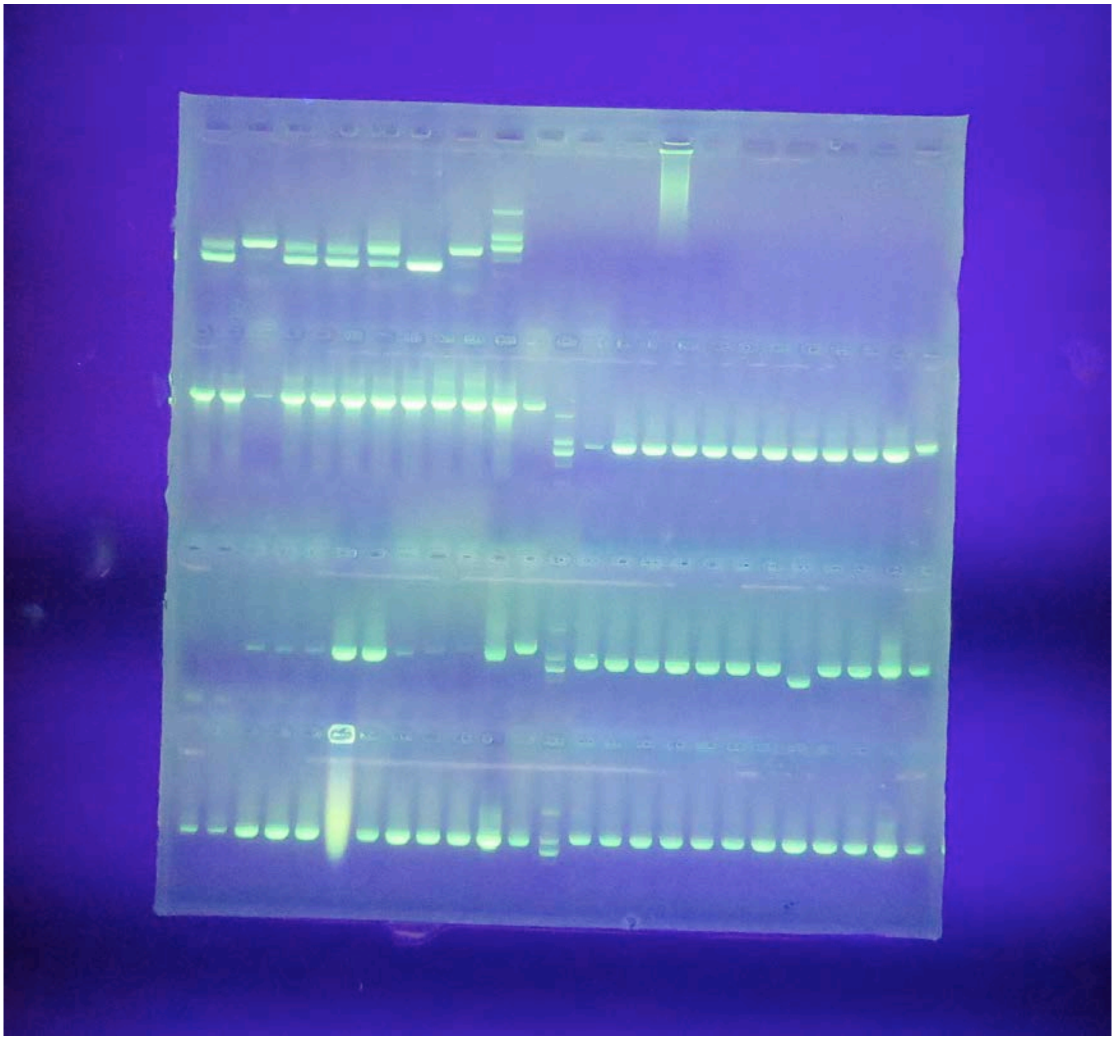
Uncropped gel of supplementary figure 10b



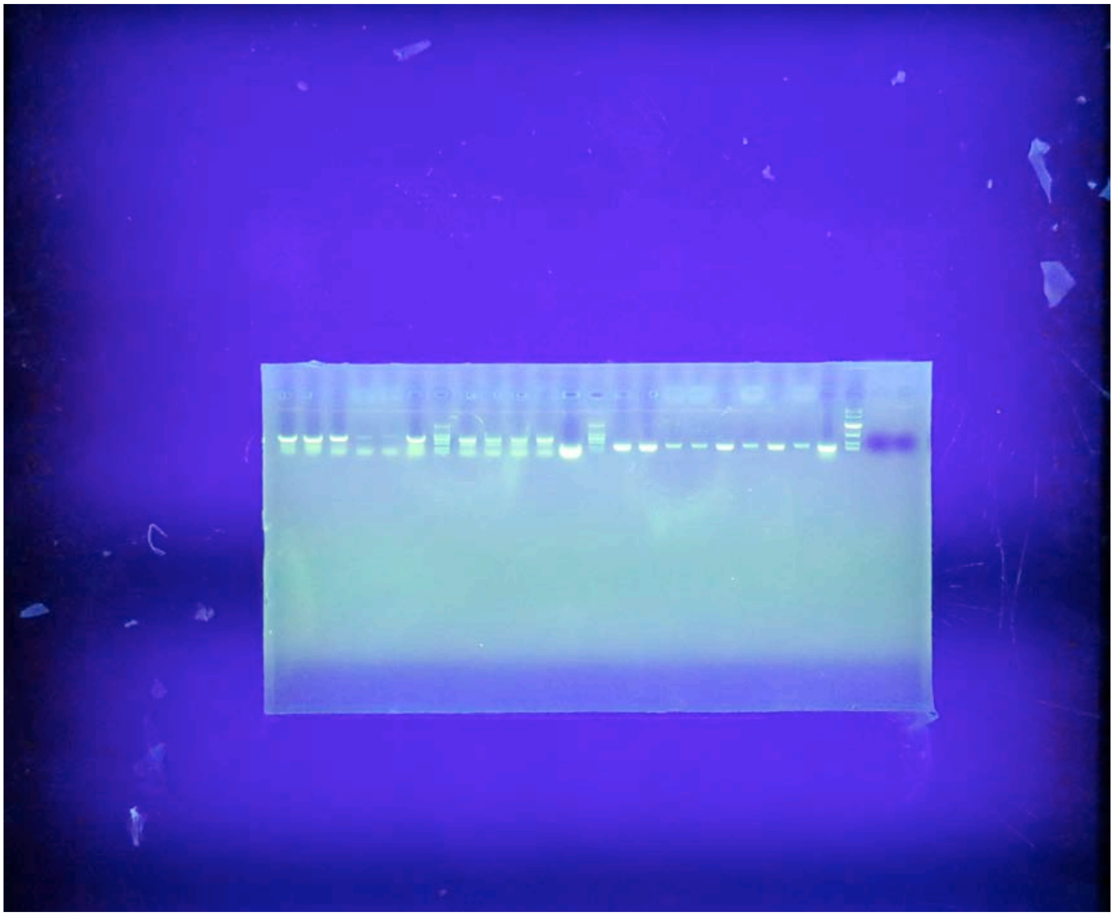
Uncropped gel of supplementary figure 10b



Uncropped gel of supplementary figure 10c



Uncropped gel of supplementary figure 13a



Uncropped gel of supplementary figure 13a