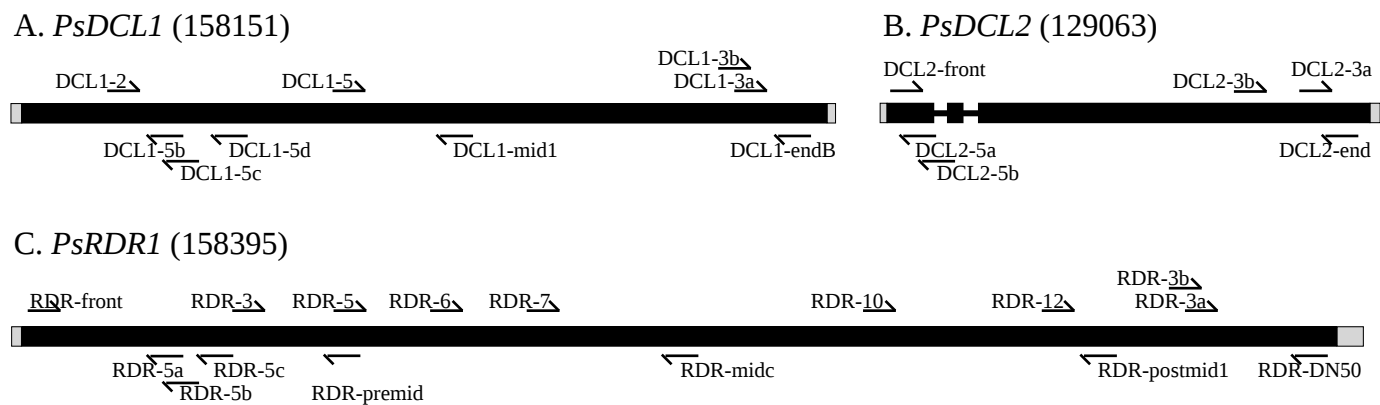




Supplementary Figure 1. Primers for cloning *P. sojae* small RNA biogenesis components (DCL and RDR). Genes are labeled followed by their Gene ID from the Eumicrobe Database. In the genomic DNA diagrams, exons and introns are represented as black bars and lines, respectively. 5' and 3' UTRs are represented as grey bars. (A-C). Positions of primers used for cloning DCL1, DCL2, and RDR from cDNA, as detailed in Supplementary Table 1.

A. Transcription start site

GCTCATTYBNNNWTTY – consensus

TGTCACTTGCATTTTT – DCL1 52b 5' UTR (major)

CATCATTTCCGCAATT – DCL1 18b 5' UTR (minor)

TCTCATTCCGTGATTT – DCL2 33b 5' UTR

TCTCACTCCCAGATTT – RDR 32b 5' UTR

B. Translation start site

ACCATGA – consensus

GCCATGG – DCL1

GTGATGG – DCL2

GCGATGA – RDR

C. Exon/intron boundaries

GTRNGT...YAG – consensus

GTAAAGC...AAG – DCL2 intron 1 (71b)

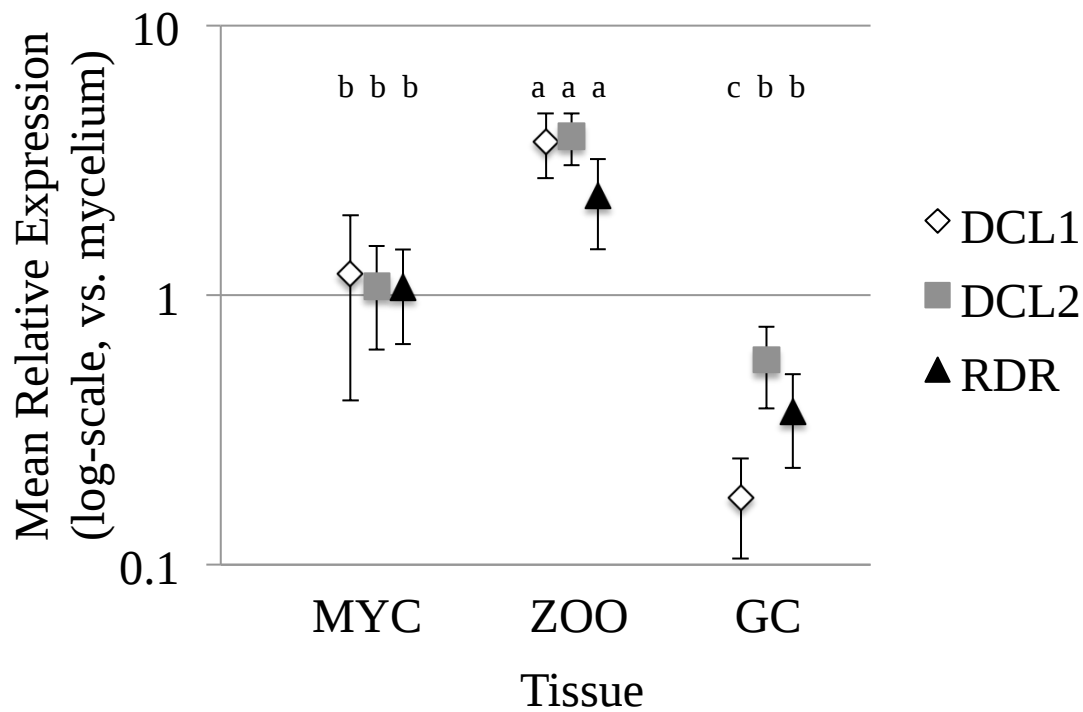
GTGAGC...CAG – DCL2 intron 2 (83b)

Supplementary Figure 2. Gene structure consensus sequence comparisons of DCL1, DCL2, and RDR.

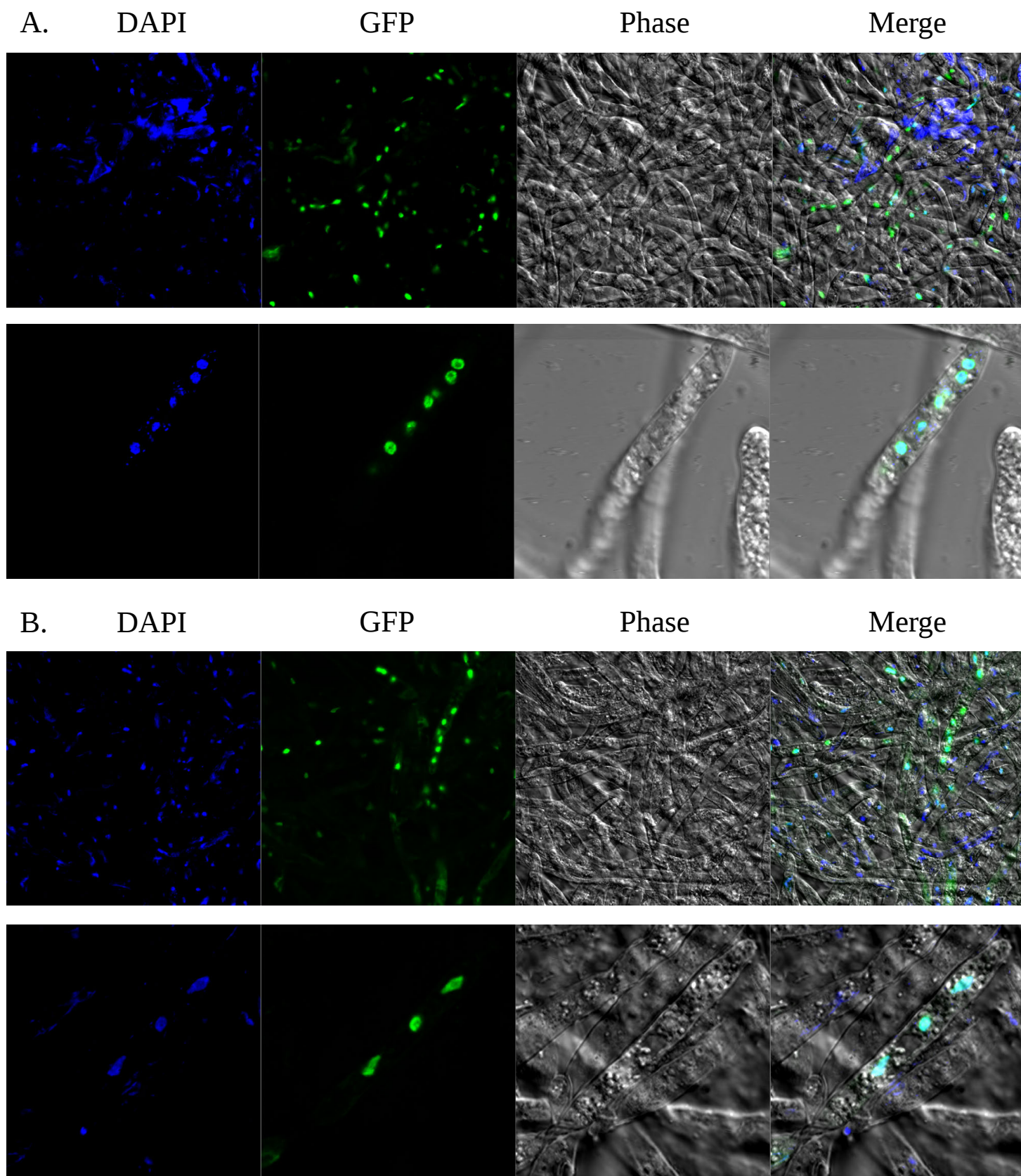
Transcription start site (A), translation start site (B), and exon/intron boundary (C) sequences for DCL1,

DCL2, and RDR are compared against the *Phytophthora* consensus sequence as described by Kamoun (2003).

Underlined bases indicate: (A) first base of cDNA, (B) translation start codon, and (C) first and last bases of intron.



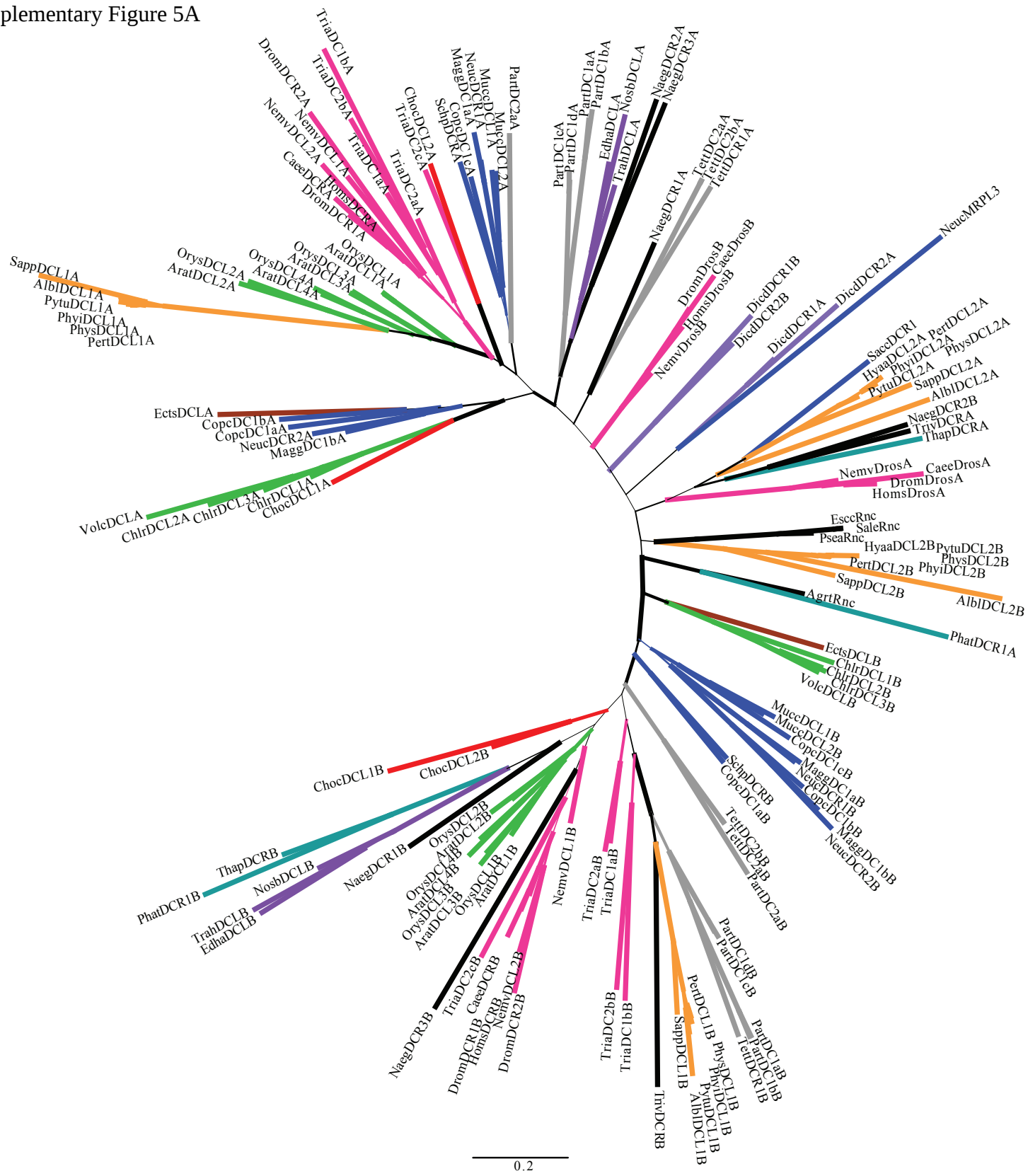
Supplementary Figure 3. Mean relative expression of DCL and RDR homologs. Mean expression of DCL1, DCL2 and RDR relative to mycelium and normalized by reference genes WS41 and β -Tubulin. RT-qPCR data is based on 6 biological replicates. MYC: mycelium; ZOO: zoospores; GC: germinated cysts. Labels a/b/c across individual genes indicate significantly different means based on Tukey's test ($p < 0.05$; shown are means and standard deviations).



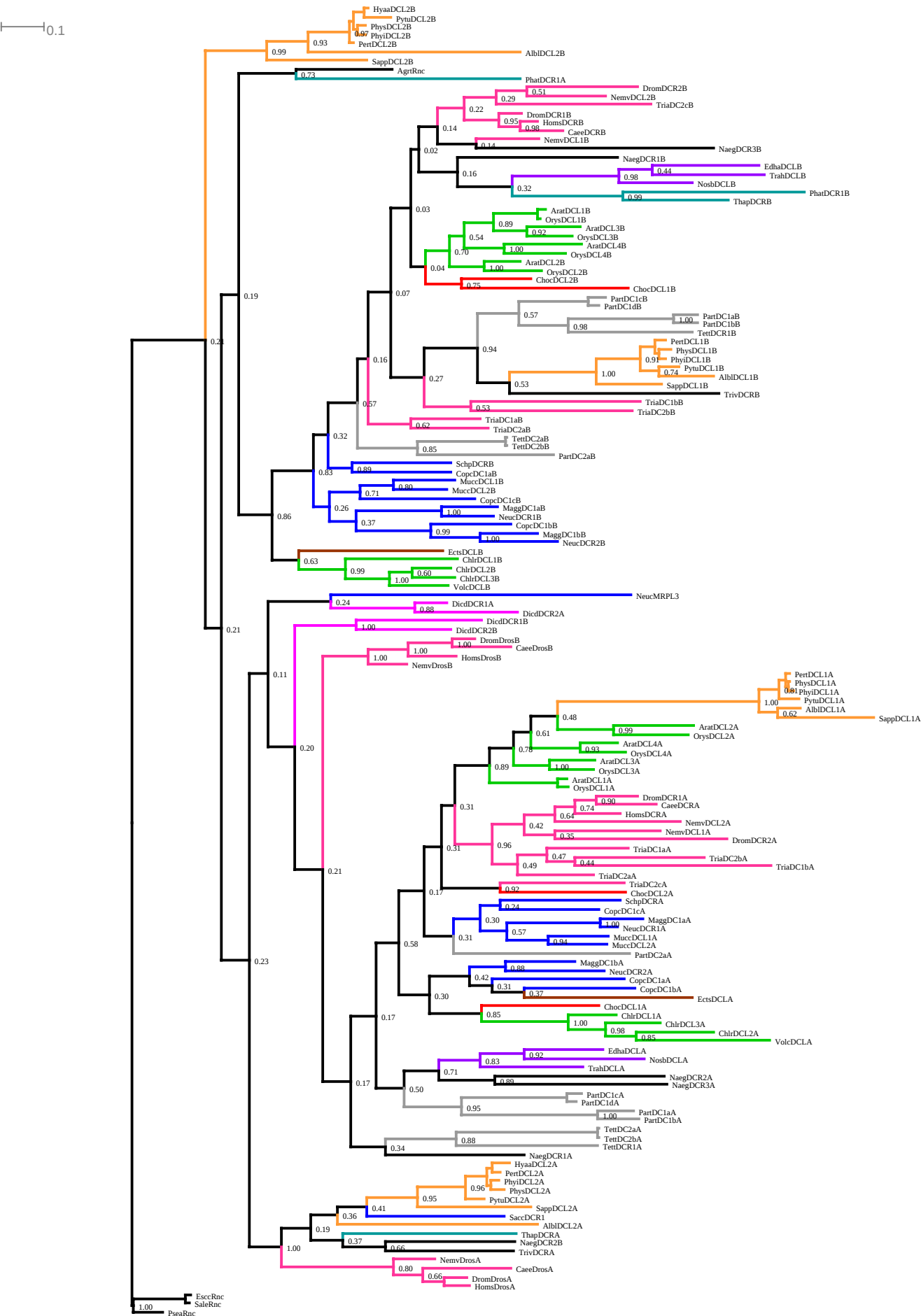
Supplementary Figure 4. Additional representative images of DCL subcellular localization in *P. sojae*.
 (A) Subcellular localization of PsDcl1; (B) Subcellular localization of PsDcl2.

Supplementary Figure 5. Consensus tree of Dicer homologs based on RNaseIIIa and RNaseIIIb domains individually. (A) Radial tree. Significance of Bayesian support is indicated as thickness of branches. (B) Rectangular tree. Bayesian support values are shown next to nodes. (A,B) Branches are colored to denote major species groups, as in Figures 2, 3, 4 and 5. Species abbreviations are defined in Supplementary Table 2A.

Supplementary Figure 5A



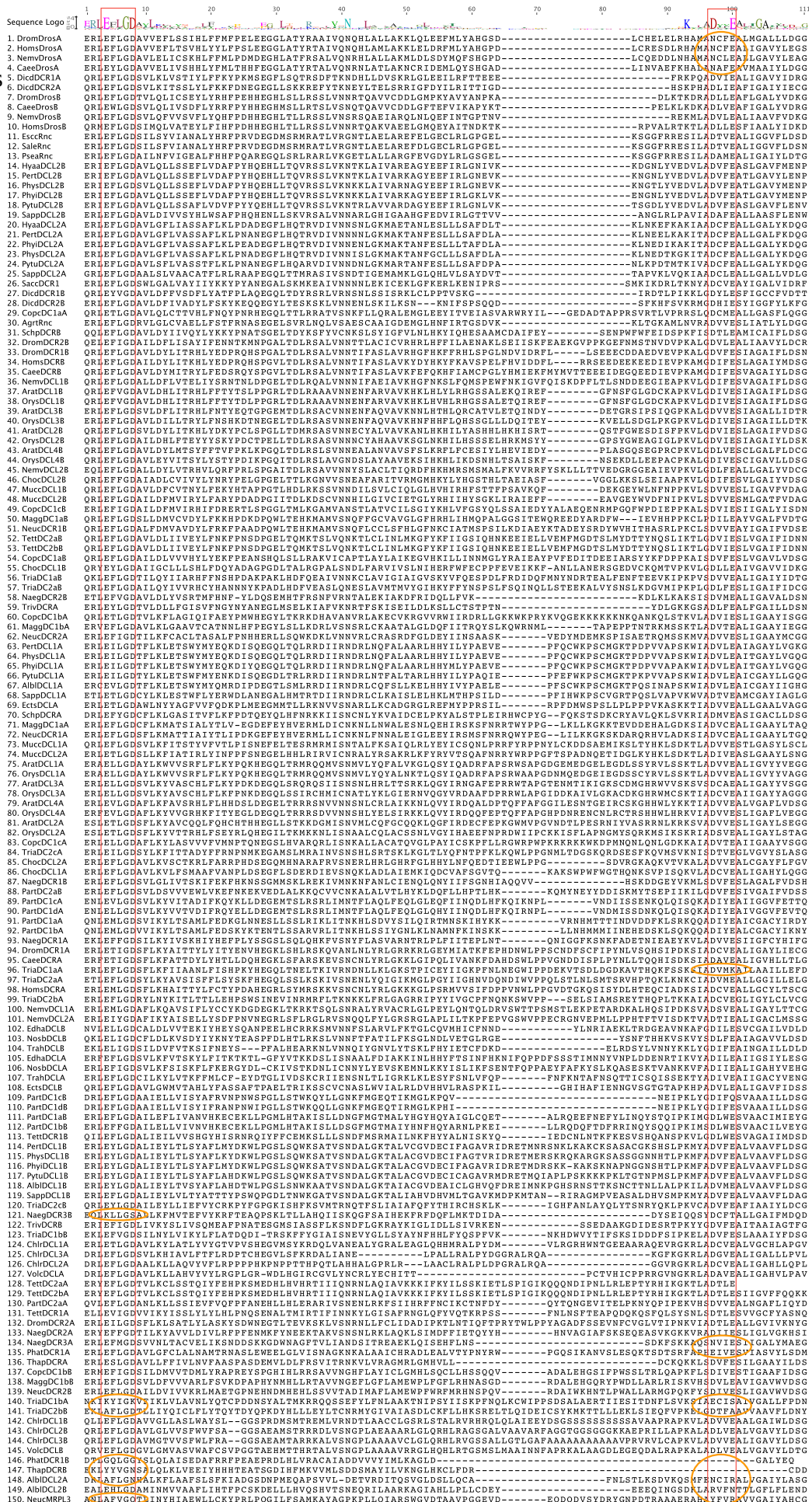
Supplementary Figure 5B

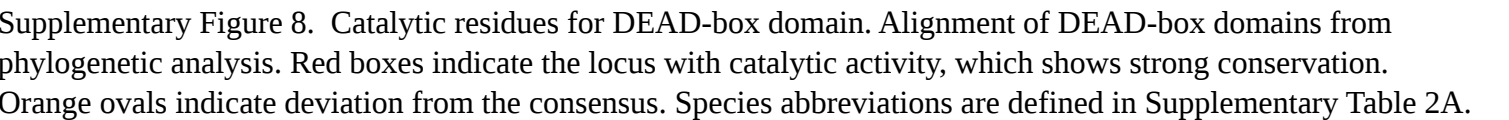


Supplementary Figure 6. Catalytic residues for RNEase III domain. Alignment of RNEase III domains from phylogenetic analysis. Red boxes indicate the first and second loci with catalytic activity, which show strong conservation. Orange ovals indicate deviation from the consensus. Species abbreviations are defined in Supplementary Table 2A.

indicate deviation from the consensus.

Species abbreviations are defined in Supplementary Table 2A.





	ATP-binding residues		Mg ²⁺ -binding residues	
	DCL1	RDR	DCL1	RDR
Consensus	GxGKT	GxGKT	DE(C/A)H	DE(C/A)H
<i>P. infestans</i>	PVGKT	GCGKT	EDWD	DEAH
<i>P. sojae</i>	PVGKS	GCGKT	EDWD	DEAH
<i>H. arabidopsidis</i>	PVGKS	GCGKT	EDWD	DEAH
<i>P. tabacina</i>	PVGKT	GCGKT	EDWD	DEAH
<i>P. ultimum</i>	RVGKT	GAGKT	EETE	DEAH
<i>A. laibachii</i>	KIGKT	GCGKT	NCCQ	ERSH
<i>S. parasitica</i>	RIGKT	--	KCCE	--
<i>E. siliculosus</i>	GVGKT	--	DECH	--

Supplementary Figure 9. Conservation of key residues in the DEAD-box helicase domain. Amino acid sequences of the Oomycete DCL1 and RDR homologs at the ATP-binding and Mg²⁺-binding sites.

Supplementary Table 1. Primers used for cloning

Forward Primer Name	Forward Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Target Gene	Gene position cloned
5'RACE forward	CGACTGGAGCACGAGGACACTGA	DCL1-5d	AAGGACGCCGACGACAGCACCGGCAGCA	PsDCL1	TSS – 678
5'RACE nested forward	GGACACTGACATGGACTGAAGGAGTA	DCL1-5b	GCGGGTTTCGTACCGGGCTCCAGTCCAT	PsDCL1	TSS – 623
5'RACE nested forward	GGACACTGACATGGACTGAAGGAGTA	DCL1-5c	TCAGCAGCGGGTTCGTACCGGGCTCCA	PsDCL1	TSS – 629
DCL1-F	ATGGAGGCCGCGCTGTGG	DCL1-R	GTCTTCCTCATCACTCGAGAACGCC	PsDCL1	ATG – term
DCL1-2	GACACGCTGCGGATCTTCG	DCL1-mid1	ATCGTCGTGGACCATTCCTC	PsDCL1	551 – 2796
DCL1-5	CACGGGTGCGATTTGAACG	DCL1-endB	CTCATCACCGCCGTGTGCC	PsDCL1	2050 – 5038
DCL1-3b	GCAGAAGCCGAGGGGCAAGAGCGCGTCA	3'RACE reverse	GCTGTCAACGATACGCTACGTAACG	PsDCL1	4807 – term
DCL1-3a	GGCGAGGGGCAAGAGCGCGTCAAGTGGA	3'RACE nested reverse	CGCTACGTAACGGCATGACAGTG	PsDCL1	4813 – term
5'RACE forward	CGACTGGAGCACGAGGACACTGA	DCL2-5b	GCGCGGGTTTATCTCGGCCTCCCATTGC	PsDCL2	TSS – 410
5'RACE nested forward	GGACACTGACATGGACTGAAGGAGTA	DCL2-5a	CATGGGCGGTGCTTGTGGCGGGTTGGCT	PsDCL2	TSS – 195
DCL2-F	ATGGACGAAGTGTCGTTGGGCTC	DCL2-R	GTCTTCGTGCAGTTTGACGCCGTAG	PsDCL2	ATG – term
DCL2-front	GAGATGGAGCACGAGAACG	DCL2-end	GGCATCCTCCTCTGAATCC	PsDCL2	67 – 2815
DCL2-3b	GCCGTTTTGTGCTGCGCTTCTGGCGTGGA	3'RACE reverse	GCTGTCAACGATACGCTACGTAACG	PsDCL2	2184 – term
DCL2-3a	CGGCAGTGGATGAACCCGCGCAAGGTGT	3'RACE nested reverse	CGCTACGTAACGGCATGACAGTG	PsDCL2	2657 – term
5'RACE forward	CGACTGGAGCACGAGGACACTGA	RDR-5c	GGGCGTCACCACCACCGCATCAAAGCCT	PsRDR	TSS – 1001
5'RACE nested forward	GGACACTGACATGGACTGAAGGAGTA	RDR-5a	CGTCGCGCGTGTAGTTCGCCCATGCTGT	PsRDR	TSS – 950
5'RACE nested forward	GGACACTGACATGGACTGAAGGAGTA	RDR-5b	CGCTCGTCGCGCTGTAGTTCGCCCAT	PsRDR	TSS – 954
RDR-front	CGAGCGACCTGGCGTGG	RDR-premid	AATCCGTCCCTGTCGAAAC	PsRDR	73 – 2051
RDR-3	CTGGAAACCACCTTACTGC	RDR-midc	TTAATCCCAAGGTCGCTCAG	PsRDR	1087 – 4135
RDR-5	CGTACCTGAATGCTCACTGG	RDR-midc	TTAATCCCAAGGTCGCTCAG	PsRDR	2093 – 4135
RDR-6	TGTCGTTCAATCTGCTGTCC	RDR-midc	TTAATCCCAAGGTCGCTCAG	PsRDR	2581 – 4135
RDR-7	TGGTGTGAAGTCCGTGAAA	RDR-postmid1	ATCAAGCCGACACATTCCC	PsRDR	3059 – 6064
RDR-10	AATGCACTGGAGGGGATCTA	RDR-postmid1	ATCAAGCCGACACATTCCC	PsRDR	4651 – 6064
RDR-12	GAAGAACTGCTGGCTGATTTG	RDR-DN50	CTCCGGGTCTGATGCACTTA	PsRDR	5652 – 8209
RDR-3b	TGAAGACAGCGGTGGCGGTGGCGGTGGT	3'RACE reverse	GCTGTCAACGATACGCTACGTAACG	PsRDR	7325 – term
RDR-3a	AGACAGCGGTGGCGGTGGCGGTGGTTCA	3'RACE nested reverse	CGCTACGTAACGGCATGACAGTG	PsRDR	7328 – term

Supplementary Table 2A. Species included in Dicer phylogenetic analyses

Supergroup	Kingdom	Phylum/subgroup	Class/subgroup	Species Name
Archaeplastida	Plantae	Angiosperms	Monocots	Oryza sativa
			Rosids	Arabidopsis thaliana
		Chlorophyta	Chlorophyceae	Chlamydomonas reinhardtii
				Volvox carteri
		Rhodophyta	Florideophyceae	Chondrus crispus
Chromalveolata	Alveolata	Ciliophora	Ciliata	Paramecium tetraurelia
			Oligohymenophorea	Tetrahymena thermophila
	Stramenopile	Heterokontophyta	Bacillariophyceae	Phaeodactylum tricornutum
			Coscinodiscophyceae	Thalassiosira pseudonana
			Oomycetes	Phytophthora infestans
				Phytophthora sojae
				Hyaloperonospora arabidopsidis
				Peronospora tabacina
				Pythium ultimum
				Albugo laibachii
				Saprolegnia parasitica
			Phaeophyceae	Ectocarpus siliculosus
Excavata	Discicristates	Percolozoa	Heterolobosea	Naegleria gruberi
	Fornicata	Metamonada	Parabasalia	Trichomonas vaginalis
Unikonts	Amoebozoa	Mycetozoa	Dictyostelia	Dictyostelium discoideum
	Animalia	Arthropoda	Insecta	Drosophila melanogaster
		Chordata	Mammalia	Homo sapiens
		Cnidaria	Anthozoa	Nematostella vectensis
		Nematoda	Secernentea	Caenorhabditis elegans
		Placozoa	Tricoplacia	Trichoplax adhaerens
	Fungi	Ascomycota	Ascomycetes	Neurospora crassa
			Saccharomycetes	*Saccharomyces castellii
			Schizosaccharomycetes	Schizosaccharomyces pombe
			Sordariomycetes	Magnaporthe grisea
		Basidiomycota	Agaricomycetes	Coprinopsis cinerea
		Microsporidia	Apansporoblastina	Nosema bombycis
			Microsporidia incertae sedis	Edhazardia aedis
			Pansporoblastina	Trachipleistophora hominis
		Zygomycota	Zygomycetes	Mucor circinelloides
Bacterial outgroups used for RNaseIII domain analysis				
Bacteria	Eubacteria	Proteobacteria	Alphaproteobacteria	*Agrobacterium tumefaciens
			Gammaproteobacteria	*Escherichia coli
				*Salmonella enterica
				*Pseudomonas aeruginosa

* outgroups for RNaseIII domain analysis only; genes only had one predicted RNaseIII domain

Supplementary Table 2B. Species included in RDR phylogenetic analysis

Supergroup	Kingdom	Phylum/subgroup	Class/subgroup	Species Name	#RDR	# with DEAD
Archaeplastida	Plantae	Angiosperms	Monocots	Oryza sativa	5	
			Rosids	Arabidopsis thaliana	6	
		Chlorophyta	Chlorophyceae	Coccomyxa subellipsoidea	1	
				Volvox carteri	1	
Chromalveolata	Alveolata	Apicomplexa	Conoidasida	Neospora caninum	1	
				Toxoplasma gondii	1	
		Ciliophora	Ciliata	Paramecium tetraurelia	2	
			Oligohymenophorea	Tetrahymena thermophila	1	
	Hacrobia	Haptophyta	Prymnesiophyceae	Emiliana huxleyi	2	
	Stramenopile	Heterokontophyta	Bacillariophyceae	Phaeodactylum tricornutum	1	
			Coscinodiscophyceae	Thalassiosira pseudonana	4	
			Oomycetes	Phytophthora infestans	1	1
				Phytophthora sojae	1	1
				Hyaloperonospora arabidopsidis	1	1
				Peronospora tabacina	1	1
				Pythium ultimum	1	1
				Albugo laibachii	1	1
				Saprolegnia parasitica	5	
				Saprolegnia declina	4	
			Phaeophyceae	Ectocarpus siliculosus	2	
Excavata	Discicristates	Percolozoa	Heterolobosea	Naegleria gruberi	10	
Excavata	Fornicata	Metamonada	Eopharyngia	Giardia lamblia	1	
Unikonts	Amoebozoa	Amoebozoa	Archamoebae	Entamoeba dispar	1	
				Entamoeba histolytica	1	
		Mycetozoa	Dictyostelia	Dictyostelium discoideum	3	3
	Animalia	Arthropoda	Arachnida	Ixodes scapularis	4	
		Cnidaria	Anthozoa	Nematostella vectensis	4	1
			Hydrozoa	Hydra magnipapillata	3	3
		Nematoda	Secernentea	Caenorhabditis elegans	4	
	Fungi	Ascomycota	Ascomycetes	Neurospora crassa	3	
			Schizosaccharomycetes	Schizosaccharomyces pombe	1	
		Basidiomycota	Agaricomycetes	Coprinopsis cinerea	6	
		Microsporidia	Apansporoblastina	Vittaforma corneae	1	
			Microsporidia incertae sedis	Edhazardia aedis	1	
			Pansporoblastina	Trachipleistophora hominis	1	
				Vavraia culicis	1	
		Zygomycota	Zygomycetes	Mucor circinelloides	3	