

**Current Biology, Volume 29**

**Supplemental Information**

**Intracellular Infection of Diverse**

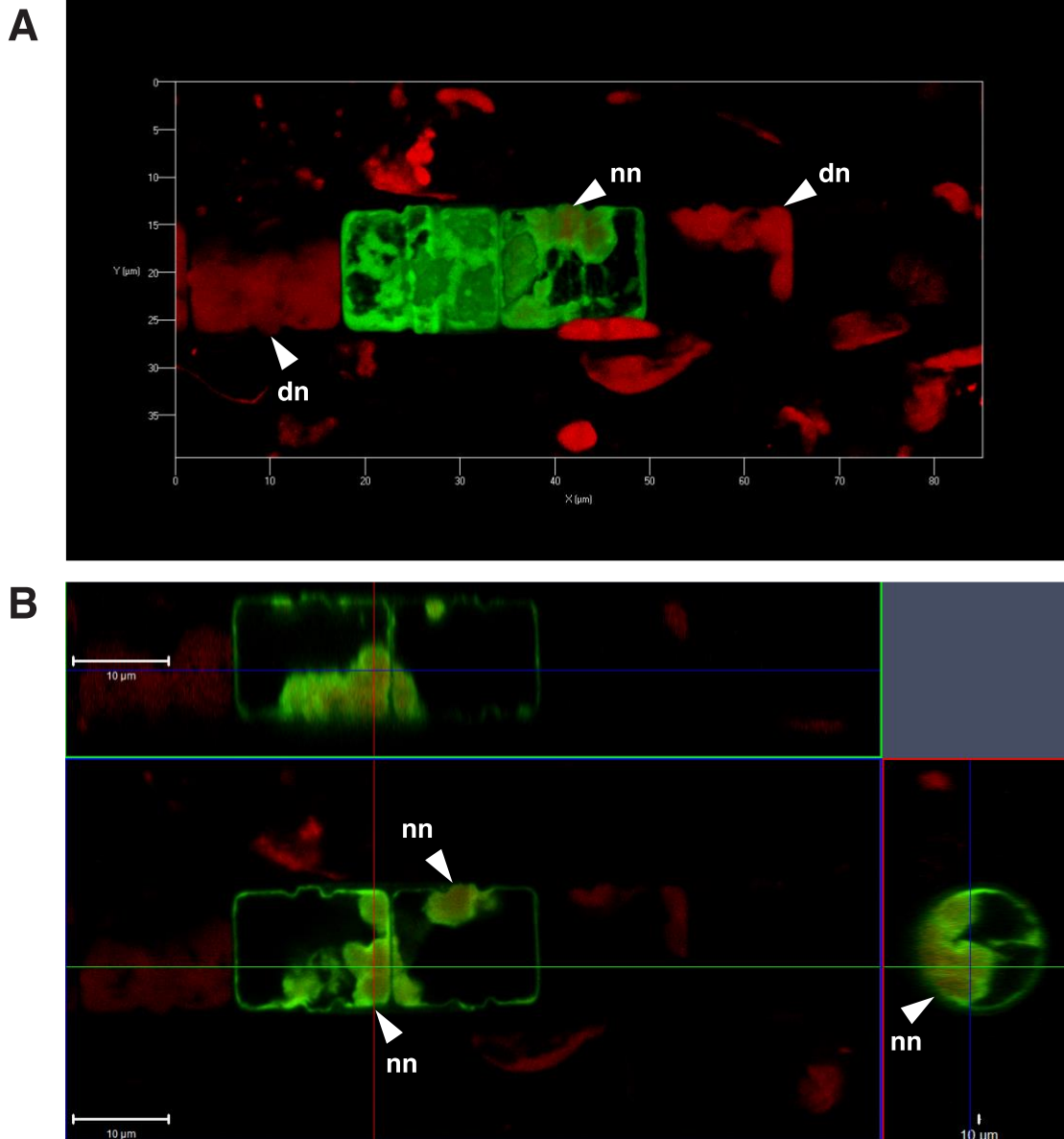
**Diatoms by an Evolutionary**

**Distinct Relative of the Fungi**

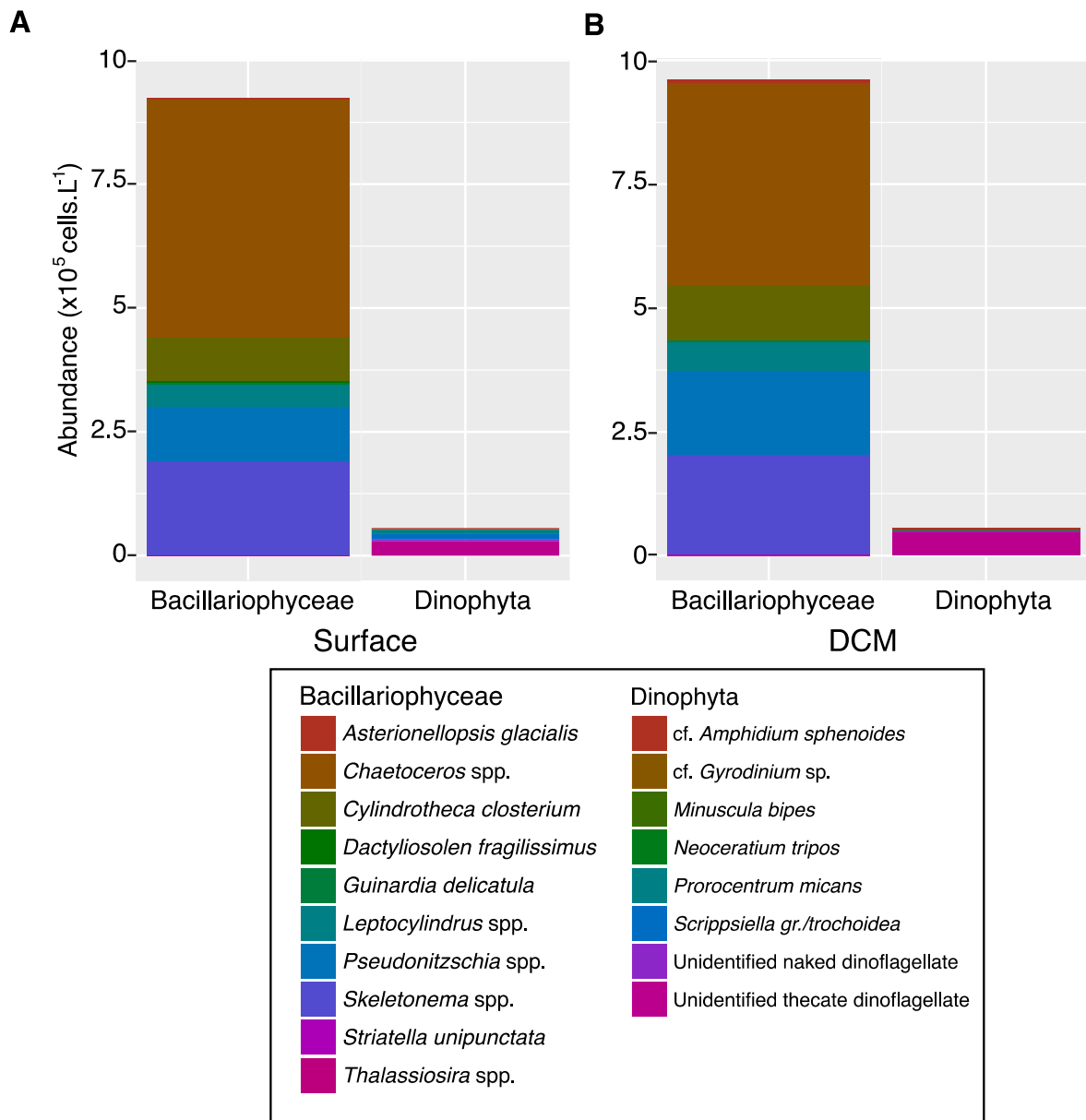
**Aurélie Chambouvet, Adam Monier, Finlay Maguire, Sarah Itoiz, Javier del Campo, Philippe Elies, Bente Edvardsen, Wenche Eikreim, and Thomas A. Richards**



**Figure S1: Phylogeny of the Chytrid-like SSU rDNA sequences with the specificity of each FISH probe used in this study. Related to Figure 1B.** Phylogenetic parameters used in this analysis are detailed in Figure 1. The grey circle represents the minimum number of mismatches necessary to obtain an identical hit between the sequences in the tree and the reverse complement of each probe (i.e. a grey circle in line with 0, demonstrates a probe will bind with no mismatches). The minimum number of mismatches is indicated on the top with right along with the probe name. The primary target (NCLC1-cluster 445) which was previously shown to be relatively abundant compared to other fungal sequences in these environmental samples is shown in red and the probe specificity score is indicated with an arrow.



**Figure S2: 3D Confocal image of intracellular infection of a *Chaetoceros* spp. -like diatom infected by NCLC1 lineage detected by Fluorescent *in situ* hybridization (FISH) from deep chlorophyll maximum (DCM, 20 meters depth). Related to Figure 2F.** Green fluorescence represents the parasitic probes (CHY-NCLC-01) targeting the small subunit ribosomal RNA (SSU rDNA) and the red fluorescence shows nuclear genomic DNA labelled by propidium iodide. (A) 3D confocal reconstruction micrograph and (B) is derived from the same cell as A but is a set of three single section views of the FISH microscopy (i.e. confocal images with orthogonal views: X, Y, Z), each section is indicated by the red, blue and green lines through each relevant image. Micrographs were obtained using Confocal Zeiss LSM780 microscope. Arrow heads labelled 'dn' and 'nn' marked putative diatom and NCLC1 nuclei respectively.



**Figure S3: Planktonic diatom and dinoflagellate species abundances (cells L<sup>-1</sup>) determined by microscopy allowing us to assay putative host diversity. Related to Figure 2G. A.** sub-surface (1 m depth) and **B.** deep chlorophyll maximum (DCM, 20 m depth) of the Oslofjorden 2009 sampling station counted from 10 ml Lugol's fixed samples collected during the same sampling expedition as for samples used for FISH.

Division/Class	Genus	Species	Abundance (Cells.L <sup>-1</sup> )		
			Surface	DCM	
Bacillariophyceae	<i>Cylindrotheca</i>	<i>closterium</i>	89400	105800	
	<i>Skeletonema</i>	spp.	189800	199400	
	<i>Skeletonema</i>	<i>marinoi</i>	-	-	
	<i>Skeletonema</i>	<i>pseudocostatum</i>	-	-	
	<i>Ditylum</i>	<i>brightwellii</i>	-	800	
	<i>Dactyliosolen</i>	<i>fragilissimus</i>	2400	-	
	<i>Thalassionema</i>	<i>nitzschioides</i>	-	-	
	<i>Leptocylindrus</i>	<i>minimus</i>	4800	4400	
	<i>Leptocylindrus</i>	<i>danicus</i>	40600	54000	
	<i>Guinardia</i>	<i>delicatula</i>	4400	3400	
	<i>Pseudonitzschia</i>	sp. 1	49800	138600	
	<i>Pseudonitzschia</i>	sp. 2	55600	29200	
	<i>Pseudonitzschia</i>	<i>calliantha</i>	-	-	
	<i>Pseudonitzschia</i>	<i>australis</i>	-	-	
	<i>Pseudonitzschia</i>	spp.	-	-	
	<i>Rhizosolenia</i>	cf. <i>pungens</i>	400	2000	
	<i>Chaetoceros</i>	<i>simplex</i>	600	1400	
	<i>Chaetoceros</i>	<i>subtilis</i>	1000	600	
	<i>Chaetoseros</i>	<i>minimus</i>	17600	-	
	<i>Chaetoseros</i>	<i>tenuissimus</i>	12400	1000	
	<i>Chaetoceros</i>	<i>wighamii</i>	25200	18200	
	<i>Chaetoceros</i>	<i>curvisetus</i>	44200	15200	
	<i>Chaetoceros</i>	<i>didymus</i>	-	-	
	<i>Chaetoceros</i>	cf. <i>pendulus</i> or cf. <i>peruvianus</i>	8000	800	
	<i>Chaetoceros</i>	<i>socialis</i>	212000	190000	
	<i>Chaetoceros</i>	<i>teres</i>	15600	600	
	<i>Chaetoceros</i>	cf. <i>brevis</i>	-	-	
	<i>Chaetoceros</i>	cf. <i>constrictus</i>	-	-	
	<i>Chaetoceros</i>	<i>danicus</i>	400	600	
	<i>Chaetoceros</i>	<i>debilis</i>	-	-	
	<i>Chaetoceros</i>	<i>decipiens</i>	8000	10200	
	<i>Chaetoceros</i>	<i>contortus</i>	600	800	
	<i>Chaetoceros</i>	spp.	138000	176400	
	<i>Asterionellopsis</i>	<i>glacialis</i>	3200	6800	
	<i>Striatella</i>	<i>unipunctata</i>	-	2400	
	<i>Coscinodiscus</i>	spp.	200	-	
	<i>Thalassiosira</i>	spp.	600	1000	
	Dinophyta	<i>Neoceratium</i>	<i>tripos</i>	200	-
		<i>Neoceratium</i>	<i>furca</i>	-	-
		<i>Prorocentrum</i>	<i>minimum</i>	-	-
<i>Prorocentrum</i>		<i>micans</i>	8800	800	
<i>Protoperdinium</i>		spp.	-	-	
<i>Minuscula</i>		<i>bipes</i>	200	-	

	<i>Scrippsiella</i>	<i>gr./trochoidea</i>	10800	600
	Unidentified	thecate dinoflagellate	29400	48400
	cf. <i>Azadinium</i>	<i>spinosum</i>	-	-
	<i>Heterocapsa</i>	<i>triquetra</i>	-	-
	cf. <i>Gyrodinium</i>	sp.	200	1200
	cf. <i>Amphidium</i>	<i>sphenoides</i>	2600	4900
	cf. <i>Amphidium</i>	<i>longum</i>	200	-
	Unidentified	naked dinoflagellate	3600	1200
	<i>Gyrodinium</i>	<i>fusiforme</i>	-	-
	<i>Karenia</i>	cf. <i>mikimotoi</i>	-	-
Haptophyta	<i>Phaeocystis</i>	<i>globosa</i>	-	400
	<i>Emiliana</i>	<i>huxleyi</i>	-	-
	<i>Chrysochromulina</i>	sp.	-	-
	<i>Chrysochromulina</i>	<i>ericina</i>	-	-
	<i>Chrysochromulina</i>	<i>polylepis</i>	-	-
	<i>Chrysochromulina</i>	<i>mactra</i>	-	-
	<i>Phaeocystis</i>	sp.	-	-
	<i>Imantonia</i>	<i>rotunda</i>	-	-
	cf. <i>Anoplosolenia</i>		-	-
	<i>Acanthoica</i>	<i>quadrispina</i>	-	-
Dictyochophyceae	<i>Dictyocha</i>	<i>speculum</i>	200	400
	<i>Apedinella</i>	<i>spinifera</i>	-	-
Cryptophyta	<i>Hemiselmis</i>	sp.	-	-
	cf. <i>Cryptaulax</i>		-	-
Chrysophyceae	<i>Meringosphaera</i>	<i>mediteranea</i>	-	-
	<i>Paraphysomonas</i>	sp.	-	-
Euglenophyta	<i>Eutreptiella</i>	sp.	200	-
Prasinophyceae (sensu lato)	<i>Micromonas</i>	sp.	-	-
	<i>Pyramimonas</i>	<i>grossi</i>	-	-
	<i>Mantoniella</i>	<i>squamata</i>	-	-
	<i>Bathycoccus</i>	<i>prasinus</i>	-	-
	cf. <i>Cymbomonas</i>		-	-
	cf. <i>Pterosperma</i>		-	-
Other	Pollen		800	-
	Ciliates		4200	1000
	<i>Paulinella</i>	spp.	-	-
	Choanoflagellate 1		-	-
	Choanoflagellate 2		-	-

**Table S1: Planktonic species abundances allowing us to assay putative host diversity. Related to Figure 2G.** These metrics were sampled from sub-surface (1 m depth) and DCM (20 m depth) samples taken as part of the BioMarKs sampling cruise at station OF (59.253735N, 10.710908E) in outer Oslofjorden, Norway on 22<sup>nd</sup> September 2009. Cells were counted in 10 mL Lugol's fixed samples.

Parasitic life stage	Probe name	Subsurface		DCM	
		% of FISH positive cells	s.d.	% of FISH positive cells	s.d.
Free living	CHY-NCLC-01	65.71	15.96	62.55	15.17
	CHY445-01	65.80	15.78	71.81	15.55
	CHY445-02	70.77	13.49	61.09	16.01
Epibiotic extracellular associations	CHY-NCLC-01	29.09	4.80	29.73	3.97
	CHY445-01	25.47	8.46	22.49	4.98
	CHY445-02	23.94	4.27	34.04	5.95
Intracellular associations	CHY-NCLC-01	5.19	2.07	7.72	3.67
	CHY445-01	8.25	2.48	5.69	4.02
	CHY445-02	4.93	2.94	4.56	2.19
'Multinucleated' cell structure	CHY-NCLC-01	0	0	0.77	0.89
	CHY445-01	0.47	0.55	0	0
	CHY445-02	0.35	0.41	0.30	0.41

**Table S2: FISH sampling statistics. Related to Figure 4A/B.** Percentage and standard deviation (s.d.) of FISH positive cells number per probes of each parasitic life stage from subsurface (1 m depth) and DCM (20 m depth) samples taken as part of the BioMarKs sampling cruise at station OF (59.253735N, 10.710908E) in outer Oslofjorden, Norway on 22<sup>nd</sup> September 2009.



ASV ID	NCLC1 reference ID (GenBank); id%	OSD samples (n; rabund%)	Highest co-occurrence	correlation (pseudo-P)
asv676	S04B23 (AB468614); 92.8%	OSD92 (21; 0.093%)	asv111; Eukaryota, Stramenopiles, Ochrophyta, Bacillariophyta, Bacillariophyta_X, Polar-centric-Mediophyceae	0.248 (0)
asv504 6	Cluster_629 (LN827851.1); 96.02%	OSD125 (24; 0.119%)	asv1118; Eukaryota, Rhizaria, Cercozoa, Filosa-Thecofilosea, Ventrileftida, CCW10-lineage	0.106 (0)
asv544 2	Cluster_786 (LN827853.1); 96.02%	OSD102 (12; 0.033%), OSD107 (12; 0.130%), OSD108 (45; 0.210%), OSD111 (8; 0.022%), OSD148 (554; 3.921%), OSD167 (2; 0.012%)	asv382; Eukaryota, Stramenopiles, Ochrophyta, Bacillariophyta, Bacillariophyta_X, Polar-centric-Mediophyceae	0.12 (0)
asv866 4	KD14_BASS (EU154992); 97%	OSD162 (5; 0.023%)	na	na

**Table S3: NCLC1-like sequences in the Ocean Sampling Day SSU-V4 datasets and ASV co-occurrence. Related to Figure 4C.** ‘id%’ correspond to the percentage of identity between ASV and NCLC1 reference sequences. ‘n’ and ‘rabund%’ correspond respectively to the sequence number and the relative abundance in OSD samples.

Group label	Accession no.	Organism/definition
Nucleariidae/Fonticula group	AF349563	<i>Nuclearia delicatula</i>
	AF349565	<i>Nuclearia moebiusi</i>
	GQ330607	Uncultured Nucleariidae clone PR2 3E 125
	AB433328	<i>Nuclearia thermophila</i>
	AY364635	<i>Nuclearia pattersoni</i>
	GQ330608	Uncultured Nucleariidae clone PRS2 3E 65
	FJ816018	<i>Fonticula alba</i>
	AY082985	Uncultured eukaryote clone RT5iin14
	AY082985	Uncultured eukaryote clone RT5iin16
	KY113120	<i>Parvularia atlantis</i>
Apusozoa	LN576893	Uncultured eukaryote clone SICF480 N11D2 18S E
	JQ340336	<i>Fabomonas tropica</i>
	JQ340338	<i>Ancyromonas atlantica</i>
Choanoflagellida	JQ340333	<i>Planomonas bulbosa</i>
	AF174363	<i>Ancyromonas sigmoides</i>
	AF271999	<i>Monosiga ovata</i>
	AF174375	<i>Monosiga brevicollis</i>
	HQ026774	<i>Salpingoeca tuba</i>
	HQ026764	<i>Acanthocorbis unguiculata</i>
	AF084234	<i>Diaphanoeca grandis</i>
	AF272000	<i>Calliacantha</i> sp.
	AF084233	<i>Acanthoeca spectabilis</i>
	L10823	<i>Acanthocephalus unguiculata</i>
Blastocladio- /Chytridio/Mucoro- /Zoopagomycota/Dikarya	AB016012	<i>Rhopalomyces elegans</i>
	AB016011	<i>Syncephalis depressa</i>
	AB0160092	<i>Zoophagus insidians</i>
	AB016010	<i>Kuzuhaea moniliformis</i>
	AB016023	<i>Piptocephalis corymbifera</i>
	DQ367463	<i>Zygopolaris ephemeridarum</i>
	DQ367460	<i>Harpella meridiana</i>
	AF007543	<i>Spiromyces spiralis</i>
	AF007542	<i>Spiromyces minutus</i>
	DQ322626	<i>Orphella haysii</i>
	AF007539	<i>Martensiomycetes pterosporus</i>
	AF007538	<i>Linderina pennispora</i>
	AF157118	<i>Absidia glauca</i>
	AF157157	<i>Radiomyces spectabilis</i>
	AJ301862	<i>Paraglomus brasilianum</i>
	Y159053	<i>Geosiphon pyriformis</i>
	AJ306443	<i>Scutellospora calospora</i>
	AJ852608	<i>Gigaspora rosea</i>
	AJ619946	<i>Pacispora scintillans</i>
	Y176502	<i>Diversispora</i> sp.
	Z14006	<i>Entrophospora colombiana</i>
	AB032651	<i>Cryptococcus vishniacii</i>
	AB126645	<i>Symmetrospora marina</i>
	DQ363306	<i>Graphiola phoenicis</i>
	Z75578	<i>Saccharomyces cerevisiae</i>
	AB028137	<i>Penicillium expansum</i>
	AF548066	<i>Aspergillus penicillioides</i>
	EU167598	<i>Mycosphaerella stromatosa</i>
	GU214700	<i>Stigmata</i> sp.
	GU214575	<i>Pseudocercospora</i> sp.
	GU214666	<i>Passalora graminis</i>
	X54264	<i>Blastocladiella emersonii</i>
	AY635840	<i>Microallomyces dendroideus</i>
	AY635822	<i>Catenophlyctis</i> sp.

JX242609	<i>Basidiobolus meristosporus</i>
AF113413	<i>Basidiobolus haptosporus</i>
EF565163	<i>Blastocladales</i> sp.
HQ888717	<i>Urophlyctis trifolii</i>
DQ536489	<i>Physoderma maculare</i>
Y17504	<i>Hyaloraphidium curvatum</i>
AB016018	<i>Monoblepharella</i> sp.
AY635839	<i>Oedogoniomyces</i> sp.
X58724	<i>Endogone pisiformis</i>
DQ536471	<i>Endogone lactiflua</i>
DQ322624	<i>Olpidium brassicae</i>
DQ536481	<i>Cyllamyces aberensis</i>
JQ040248	<i>Gamsiella multidivariata</i>
AF113425	<i>Mortierella wolfii</i>
EU428769	<i>Zygomycete</i> sp.
HQ667504	<i>Mortierella cystojenkini</i>
AY635844	<i>Endochytrium</i> sp.
AY635835	<i>Nowakowskiella</i> sp.
AF1642782	<i>Arkaya lepida</i>
HQ901766	<i>Neokarlingia chitinophila</i>
AH009039	<i>Lacustromyces hiemalis</i>
AY601711	<i>Polychytrium aggregatum</i>
FJ822966	<i>Chytridium olla</i>
GU358606	<i>Phlyctochytrium aureliae</i>
FJ799984	<i>Zygorhizidium planktonicum</i>
DQ536484	<i>Rhizidium endosporangiatum</i>
KC812611	<i>Delfinachytrium mesopotamicum</i>
HQ219341	Uncultured Chytridiomycota clone AY2009C3
M59758	<i>Chytriomycetes hyalinus</i>
AH009055	<i>Podochytrium dentatum</i>
AH009056	<i>Obelidium mucronatum</i>
DQ536491	<i>Blyttiomycetes helicus</i>
GQ995306	Uncultured Chytridiomycota clone T5P2AeE01
AY635830	<i>Catenomyces</i> sp.
AY635829	<i>Rhizophlyctis rosea</i>
AF1642472	<i>Gaertneriomycetes semiglobifer</i>
DQ536490	<i>Spizellomycete</i> sp.
GQ499383	<i>Kochiomycetes</i> sp.
M59759	<i>Spizellomyces acuminatus</i>
JN940935	<i>Spizellomyces plurigibbosus</i>
JQ014020	<i>Phlyctochytrium arcticum</i>
DQ536480	<i>Triparticalcar arcticum</i>
EF014367	<i>Entophlyctis confervae-glomeratae</i>
HQ901746	<i>Fimicolochytrium jonesii</i>
AF164245	<i>Powellomyces</i> sp.
JN940943	<i>Spizellomyces dolichospermus</i>
FJ827650	<i>Gaertneriomycetes tenuis</i>
AY635826	<i>Entophlyctis helioformis</i>
AY032608	<i>Chytridium polysiphoniae</i>
EF100411	Uncultured eukaryote clone D5P09A02
EF432822	<i>Chytridiales</i> sp.
EF638581	Uncultured Chytridiomycota clone MV2E2 E8R
EF443137	<i>Chytridiales</i> sp.
AF164253	<i>Lobulomyces angularis</i>
EF443136	<i>Chytridiales</i> sp.
EF443135	<i>Chytriomycetes poculatus</i>
AY635824	<i>Entophlyctis</i> sp.
AB695513	Uncultured eukaryote clone MPE2-18
JF730792	Uncultured eukaryote clone Ch8A2mG4
EU154975	Unclassified marine fungus clone DB38 BASS
EU087186	Uncultured eukaryote clone 1 15
DQ244005	Uncultured fungus clone PFB1AU2004
AY349051	<i>Rhizophyidium</i> sp.

AF164272	<i>Rhizophlyctis harderi</i>
AY349048	<i>Rhizophlyctis harderi</i>
FN690503	Uncultured fungus clone 5-E11
DQ103819	Uncultured marine eukaryote clone M4 18F09
HQ866155	Uncultured eukaryote clone SGSP472
HQ219378	Uncultured Chytridiomycota
AY642742	Uncultured eukaryotic picoplankton clone A44
DQ536492	<i>Rhizophydium</i> sp.
GU568165	Uncultured soil fungus clone 8489
AH009034	<i>Rhizophydium</i> sp.
AY635821	<i>Rhizophydium</i> sp.
DQ536478	<i>Kappamyces laurelensis</i>
AY642706	Uncultured eukaryotic picoplankton
JN940937	<i>Kappamyces</i> sp.

---

**Table S4: Details of sequences/taxa present in collapsed branches. Related to Figure 1B.**