

Fig. S1. a) Red snow surface (sample 1) caused by microalgae at Kühtai, Tyrolean Alps, Austria. b) The discoloration reached several centimetres down, changing from red to green.

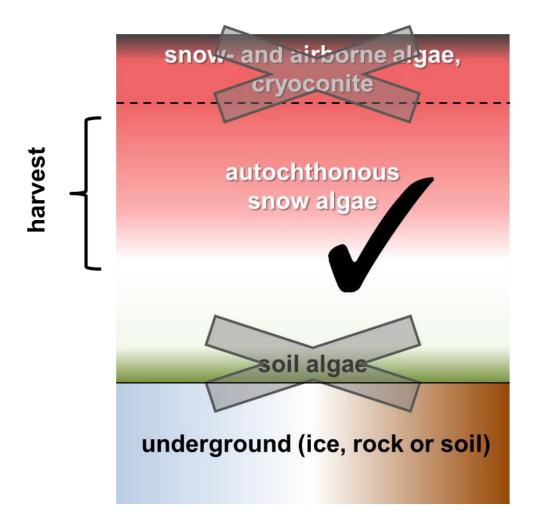


Fig. S2. Vertical scheme of a melting snowfield showing the best practice of 'true' (autochthonous) snow algae sampling. In order to avoid allochthonous organisms (airborne, soil bound), the uppermost surface and lowermost ground centimeters of snow should not be harvested. A consistent sampling strategy is important when the aim is to compare microbial diversity and abundances between different sites. Exceptions may be steep slopes in polar regions, where snow algae populations are surface based, and thus, surface layer cannot be omitted.

ACTIVITY	ном	AIM
1. selection of sequences	pairwise BLAST search: - against NCBI custom download library - against additional reference sequences	- generation of dataset of ITS2 sequences: reference sequence and all OTUs which passed the identity treshold of >= 94.0% when compared to this reference sequence
2. individual RNA structure prediction	 carry out for each of the sequences in the ITS2 dataset at server MFold: http://unafold.rna.albany.edu/?q=mfold ITS2 sequences are pasted into window at RNA Folding Form and folded note: HTS delivers DNA based data, but during RNA folding, thymin ['T'] are converted to uracil ['U'] 	 selection of the model of the secondary structure with the minimum free energy having specific features (4 helixes, U-U mismatch in helix II, UGGU motif in helix III) saving Vienna file (XFasta format, i.e. fasta file with line containing structural information in bracket-dot-bracket notation below the sequence)
3. sequence and secondary structure alignment	 paste sequences in MEGA - in each row is ITS2 rRNA sequence followed by its structural information load this *fas file into 4SALE 4SALE available here: http://4sale.bioapps.biozentrum. uni-wuerzburg.de/quickstart.html 	 performing sequence and secondary structure alignment in the first step automatically via Clustal inspection of the secondary structure (structure viewer, detection of misaligned sequences, gap, conservation, alignment column position, helix position), monitoring structure information and manual editing (in edit mode) mapping alignment to consensual structure
4. analyzing CBCs	 in homologous positions of the ITS2 molecule which are unambiguously aligned 4SALE -> Window -> CBC Table , -> Export CBC matrix inspection of positions of CBCs in consensual model of ITS2 molecule via structure viewer 	 visualisation of structural differences marked CBCs in the CBC table are highlighted in the alignment and the structure window presence of at least one CBC near the 5'-apex of helix III of ITS2 may predict a failure to sexually cross
5. secondary structure drawing	 VARNA (visualisation Applet for RNA) http://varna.lri.fr/ edit in graphic programme Inkscape https://inkscape.org/en/ 	- graphical output of secondary structure of ITS2 rRNA, and comparison nucleotide differences between OTUs assigned to the same reference sequence and visualisation of CBCs positions (if any)

Fig. S3. Schematic workflow for taxonomic assignments of environmental ITS2 OTUs from environmental samples of snow algal communities where Chlamydomonadacae prevail. For this taxonomic group the CBC species concept (in frame of a polyphasic approach) was successfully applied (e.g., MATSUZAKI et al.2015). The aim of the process is to get sequence-structure alignments of selected OTUs with their reference sequences and then search for compensatory base changes (CBCs) in homologous positions near the 5'- apex of helix III encompassing the YGGY motif (the most conserved region of the ITS2 secondary structure of eukaryotes).

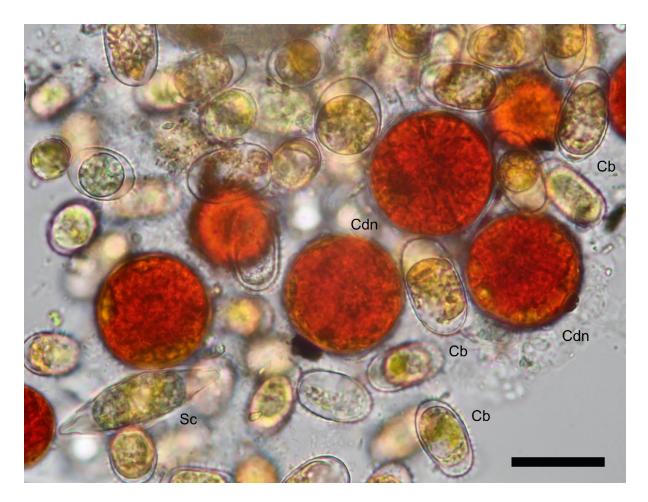


Fig. S4. Light micrograph of cells in field sample 2. The three locally abundant snow algae identified using morphological features were *Cr. brevispina* (Cb; later identified by HTS and secondary structure prediction as an undescribed species - OTU denovo99), *Scotiella cryophila* K-1 (Sc) and *Cd. nivalis* (Cdn). Scale bar: 20 µm.

10	20	30	40	50	60
GGUUAAUCGUCCCA	CCCUUUCCUG	- <mark>C-UGUGCUU</mark>	CAAACCACCG	CAG-CCA-	-AG-GA
(((((((((((((- (((())).)))))-	-))-).
GGUUAAUCGUCCCA	CCCUUUCCUG	- <mark>C-UGUGCUU</mark>	CAAACCACCG	CAG-CCA-	-AG-GA
(((((((((((((- (((())).)))))-	-))-).
GGUUAAUCGUCCCA	CCCUCUCCUG	- <mark>C-UGUGCUU</mark>	CAAACCACCG	CAG-CCG-	-AG-GA
(((((((((((((- (((())) .)))))-	-))-).
GGUUAAUCGUCCCA	CCCUUUCCUG	- <mark>C-UGUGCUU</mark>	CAAACCACCG	CAG-CCA-	-AG-GA
(((((((((((- (- (((()))))-)	-))-))
GGUUAAUCGUCCCA	UCCCAUUG	-A-U-UG-UU	CACU-	CAGUCCU-	- <mark>U</mark> G-G-
(((((((.(((- (- (- (()))))	-))-)-
GGUUCCCUGUCUCA	ACCUCCAA	CAUU-UAUUU	AA UU A	UAAUUG-UCA	GAGUGU
(((((((((((((.()))).))-)))).))
GGUUAAUCGUCCCA	UCCCAUUG	-A-U-UG-UU	CACU-	CAGUCCU-	- <mark>U</mark> G-G-
(((((((. ((((- (- (- (()))))	-))-)-
	GGUUAAUCGUCCCA (((((() GGUUAAUCGUCCCA (((()()) GGUUAAUCGUCCCA (((())) GGUUAAUCGUCCCA (((())) GGUUAAUCGUCCCA ((())) GGUUCCCUGUCUCA ((())) GGUUAAUCGUCCCA	GGUUAAUCGUCCACCCUUUCCUG (((((((((((((((((((((((((((((((GGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUU ((((((((((((((((((((((((()) GGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUU (()((((((((((((((((()) GGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUU (()((((((((((((((((((()) GGUUAAUCGUCCCAUCCAUU-G-A-U-UGUU (()((((((((((((()) GGUUAAUCGUCCCAUCCAUU-G-A-U-UGUU (()(((((((((((((())) GGUUCCUGUCUCAACCUCCA-ACAUU-UAUUU (()(((((((((((())))))))))))))))	GUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGGUUAAUCGUCCCACCCUCUCCUG-C-UGUGCUUCAAACCACCGGUUAAUCGUCCCACCCUCUCCUG-C-UGUGCUUCAAACCACCGGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGGUUCAAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGGUUCAAAUCGUCCCAUCCAUU-G-UGUGCUUCAAACCACCGGUUCAAUCGUCCCAUCCAUU-G-A-U-UG-UUCA-CU-GGUUCCCUGUCCAACCUCCA-ACAUU-UAUUUAA-U-UUAUUCAA-U-U-AUUUCAA-U-U-AUUUCAA-U-U-AUUUCAA-U-U-AUUUCAUCGUCCCAUCCAUUCCA-CU-GGUUCCUGUCCAAUCCACUCCA-ACAUU-UAUUUAA-U-UUAUUUCAA-U-U-AUUUCAA-U-U-AUUUCAUUUCAAUCGUCCAUCCAUUCCAU	10 20 30 40 50 GGUUAAUCGUCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGCAG-CCA- (())))))))) GGUUAAUCGUCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGCAG-CCA- (()((((((((((((((((((((((((((((((

	70	8	•	90	100	110	120
		•	•				
							.
Cr. brevispina	CACGUGGGGA	ICAAUCGCI	GUCUGGG	AGCUCACGCU	UC-CAGUCU	G <mark>CCC</mark> AAG <mark>U</mark>	AUUUAUU
))))))	((((.((((((()))))-))).))))	(((
denovo107	CACGUGGGGA	CAAUCGCA	GUCUGGG	AGCUCACGCU	UC-CAGUCU	G <mark>CCC</mark> AAG <mark>U</mark>	AUUUAUU
))))))	(()	((.((((((()))))-))).))))	(((
denovo249	CACGUGGGGA	ICAAUCGC/	GUCUGGG	AGCUCACGCU	UC-CAGUCU	G <mark>CCC</mark> AAG <mark>U</mark>	AUUUA-U
))))))	((((.((((((()))))-))).))))	(
denovo173	CACGUGGGGA	ICAAUCGCA	GUCUGGG	AGCUCACGCU	UC-CAGUCU	G <mark>CCCA</mark> AG <mark>U</mark>	AUUUA-U
))))))	(((. ((((((()))))-))).))))	– (
denovo99	CAUAUGGGGA	ICAAUCGCA	GUCUGGG	JG <mark>CUCACGC</mark> A	UC-CAGUCU	G <mark>CCC</mark> AAG <mark>U</mark>	AUUUAUU
))))))	(((. ((((((()))))-))).))))	(((
denovo266	C-U-UGAGGAC	CGAUGGC	GUCUGGG	CAUUUAU-UU	JGCACAGUCU	GCCCAAAU	GAC-A-U
)))))	(((((.((.((())).))).)))))	(- (
denovo248	CAUAUGGGGAU	ICAAUCGCA	GUCUGGG	JGCUCACGCA		G <mark>CCCAAGU</mark>	AUUUAUU
))))))	(()	((.((((((()))))-))).))))	(. (

	190	200	210	220	230	240
						.
Cr. brevispina	UCGAGUAUUCAUC	UCGUAUGUCU	C-UCGAA-UC	JG <mark>CC</mark> GG <mark>UUU</mark> GG	GCAAUGAAC	3 U C
	((((()	••••)))••••)-)))))).)))))).)))).)).	(((
denovo107	UCGAGUAUUCAUC	UCGUAUGUCU	C-UCGAA-UC	JGCCGGUUUGG	GCAAUGAAC	GUC
	((((()	••••)))••••)-)))))).)))))).)))).	(((
denovo249	UCGAGUAUUCAUC	UUGUAUGUCU	C-UCGACCUC-	-GACGGUUUGC	UCAAUGAA	AC
	((((()))))-)))))))))))).	.))).))	(
denovo173	UCGAGUAUUCAUC	UCGUAUGUCU	C-UCGAA-UCU	JG <mark>CC</mark> GG <mark>UUU</mark> GG	GCAAUGAAC	AUGCUUC
	((((()(((.))))-))))))).)))))).)))).	. ((((
denovo99	UCGAGUAUUCAUC	UUGUAUGUUU	AAUCGAA-UCU	JG <mark>CU</mark> GG <mark>UUU</mark> GG	GCAAUGAAC-	- UC
	((((.((((••••)))•••)).)))))))).)))))).)))).	- ((
denovo266	UCGAGUAUUCAUC	UCGUAUGUCU	C-UCGAA-UC	JG <mark>CC</mark> GG <mark>UUU</mark> GG	GCAAUGAACO	3 U C
	((((()(((.))))-))))))).)))))).)))).)).	(((
denovo248	UCGAGUAUUCAUC	UUGUAUGUUU	C-UCGAC-UU	CGACAGCUUGC	GCUCAGAUCO	CUC
	((((()(((.	••••)))••••)-)))))))))))).))))	. ((
	250	260	270	280	290	300
	••••					
Cr. brevispina						
	((((((- (-					
denovo107	UGGUGU-G-					
	((((((- (-	•••••••••••••••••••••••••••••••••••••••	-)))))))))))))).))
denovo249	G <mark>U</mark> GA-					
	((((-					
denovo173	-GG <mark>U</mark> GG <mark>C</mark> A-GAA-	UUU-AUU	UCGGUCGCC-(GUCUCAUGCAA	CCCUCCAC	CACU-U-
	- (((((((((-					
denovo99	UUGUCG-AA					
	((((.	•••••••••••••••••••••••••••••••••••••••	-))))))) -	•••••••••••••••••••••••••••••••••••••••))).))
denovo266	UGGUGU-G-	CUUCAAAC-C	-ACACCAC	3AC	:ccucccct	JAUUCAA
	((((((- (-					
denovo248	UU GA-C-A-					
	(((. - (- (-)	-))))))) -	·))))).).

	310 320
Cr. brevispina	UCCUUGRAAACCCAACACUUCU
)(((())))))
denovo107	UCCUUGGAAACCCAACACUUCU
)((((())))))
denovo249	GCAUUGGAAACCCAACACUUCU
)((((())))))
denovo173	-CAUUGGAAACCCAACACUUCU
)(((())))))
denovo99	UCCUUGGAAACCCAACACUUCU
)((((())))))
denovo266	UCCUUGGAAACCCAACACUUCU
)((((())))))
denovo248	UUAUUGGAAACCCAACACUUCU
)((((()))))))

Fig. S5. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Chloromonas brevispina* K-2 (accession number MG791868) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U']).

HQ404889 <i>Cr. pichinchae</i> denovo100 denovo85	10 . GGUUCUCAAUCACACZ ((((((() GGUUCUCAAUCACACZ ((((()()) GGUUCUCAAUCACACZ (((())))))))))))))))))))))))))))	AUCAACACU (. ((((((. AUCCAAACUC- (((((- AUCGACACU	CCU-UGUGU)))) CUUGUGAGU))))) CCU-UGUGU	JCUGUUCCUGU))))) JCUCUUUGU))) JUUGUCCCUGU	GUGGACAUCGC)))))((GUGGACAUCGC)))))((GUGGACAUCGC	CAGUC (((.(CAGUC (((.(CAGUC
HQ404889 <i>Cr. pichinchae</i> denovo100 denovo85	70 . UCUUCACCUUGUGUCC ((((()))) UCUUCACCAUGUGUCC ((((())))	GAGUCUGCCAA)).)))) GAGUCUGCCAA)).)))) GAGUCUGCCAA	AGUAUGAGU AGUAUGAGU (AGUAUGAGU	JAGCGCUUUCAJ (((((((((((((((((((AGCAUUGAAGU . (((((. ((AGCAUUGAAGU . (((((. ((AGCAUUGAAGU	JGGAC (((((JGGAU (((((JGGGC
HQ404889 <i>Cr. pichinchae</i> denovo100 denovo85	130 . CGCAAGGUCUGCCUC2 ())))))))))) CGUAAGGUCUGCUUC2 ()))))))))))) CGCAAGGUCUGCCUC2 ()))))))))))))	UUCAAGCCAC	ACCCGAGU2 (((((ACCCGAGU2 (((((ACCCGAGU2	AUGCAUUUCAAI (. ((((AUGCAUCUUUGI (. ((((AUGCAUUUCAU	UAUGUUGCUCG .))))))))))) UAUGUUGCUCG .))))))))))) UAUGUUGCUCG	BAUCU BAUCU BAUCC
HQ404889 <i>Cr. pichinchae</i> denovo100 denovo85	190 . UGUGUCUUGCUUGAUG)))).)))).)). UGUGUCUUGCUUGAUG)))).)))).)). UGUGUCUUGCCUGAUG)))).)))).))	CCAGUCUCGA	GCAAUCGAO GCAAUCAAO GCAAUCAAO GCAAUCGAO	SACAAGCAUGCU)))))))))))))) SAUAAGCAUGCU))))))))))))))))))))))))) SAUAAGCAUGCU	UGCCUAUAUUG)))((((UGCCUAUAUUG)))((((UGCCUAUAUUG	GAAC ((GAAC ((GGAAC
HQ404889 Cr. pichinchae denovo100 denovo85	250 . CCCUCAAUUCU))))).)) CCUCCAAUUCU)))))))) CCUUCAAUUCU					

Fig. S6. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Chloromonas pichinchae* (accession number HQ404889.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

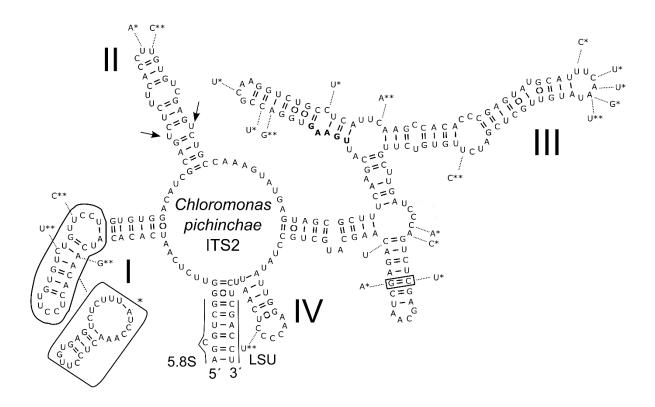


Fig. S7. Comparison of the secondary structure of ITS2 rDNA transcripts between Chloromonas pichinchae CCCryo 261-06 (accession number HQ404889.1) and the closely related OTU 'denovo100' and OTU 'denovo85'. Differences characteristic for both OTUs are shown by nucleotides outside the structure and are linked by dotted lines. One asterisk means that the difference was detected only in OTU 'denovo100', and double asterisks imply that the difference were detected in OTU 'denovo85'; middle and top part of helix I (encircled) represent an expansion segment, whose length is not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al.2013). Therefore, this part of helix I characteristic for OTU 'denovo100' is shown outside the structure and is linked by dotted lines. A single compensatory base change (CBC) in comparison with the reference sequence was found in OTU 'denovo100' but outside the most conserved part of helix III (the most conserved is close to the 5'end of III helix) and, therefore, this CBC represents intraspecific variability of Chloromonas pichinchae. OTU 'denovo85' had no CBC when compared to the reference species, and thus, was assigned to Chloromonas pichinchae. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

	10 	20	30	40	50	60
KM020046.1 L. incisa	GGUCCAUCCCCUCAC-A	ACCCGCCCUUT	JGUUUAGGC	AA-UGGGUUGG	GCUGUGGAUC	UGGC
denovo124	((((((-) GGUCCAUCCCCUCACCA					
	((. ((((()))))))))	(((
KT119889.1 L. sp.	GGUCCAUCCCCUCAC-2					
denovo28	GGUCCAUCCCCUCAC-A					
	(((((-	(((((((((((())))).)))))))))))))))))).))))	(((
	70	80	90	100	110	120
KM020046.1 L. incisa						
MADEOUAD.I I. INCIDA	((((((((.))))))).)))))	
denovo124	CGUGUCGGCUGCUGCCC (((((((((()			UGCGGCCGAGU		
KT119889.1 L. sp.	CGUGUCGGCUGC	<mark>CCCG</mark>		UGUGGCCGAGU	CGGCUGAAGC	UCAG
denovo28	((((((((((CGUGUCGGCUGC					
denovozo	((((((((((
	130	140	150	160	170	180
KM020046.1 L. incisa	AGGCCUGAGCAAGCGCC					
denovo124	AGGCCUGAGCAAGAGC					
KT119889.1 L. sp.	. (((((((((((((((((((
кнізовэлі Ш. эр.	. (((((((((((((((((((
denovo28	AGGCCUGAGCAAGCGCC					
	• • • • • • • • • • • • • • • • • • • •	((((((,,,,
	190 	200	210	220	230	240
KM020046.1 L. incisa	GAAGCCGUUGAUGGCCC					
denovo124	AUUGCCGUUGAUGACC					
denov0124)))))))))))))))))))))))))))))))))))))					
KT119889.1 L. sp.	GAAGCCGUUGAUGGCCC					
denovo28	GAAGCCGUUGAUGACC					
	•••)))))))))))))))))))))))))))))))))))))))).))))))))))))	(()	(((((())
	250	260				
KM020046.1 L. incisa						
))))).)))))				
denovo124	-GUGCCGGUGCCGAC-(
KT119889.1 L. sp.	AGGGCUGGUGGCGACU	CACCUUUC				
denovo28	.)))))))))					
461104020))))))))))					

Fig. S8. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Lobosphaera incisa* SAG 2466 (accession number KM020046.1), OTU 'denovo124', *Lobosphaera* sp. K-1 (accession number KT119889.1) and OTU 'denovo28'. No compensatory base change (CBC) was found in the dataset. Thus, both OTUs were assigned to species *Lobosphaera incisa* (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

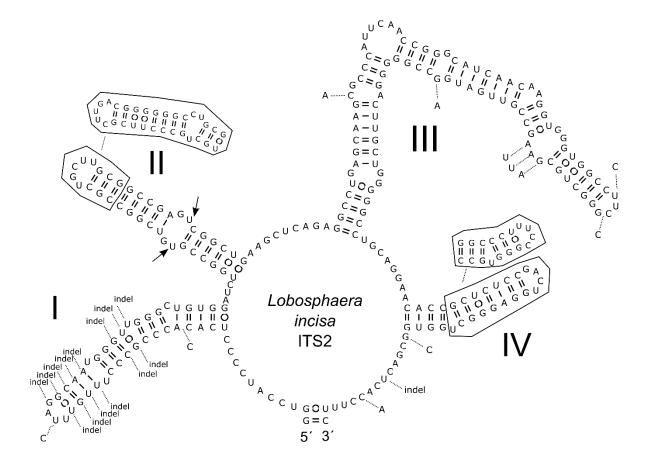


Fig. S9. Comparison of the secondary structure of ITS2 rDNA transcripts between *Lobosphaera incisa* SAG 2466 (accession number KM020046.1) and the closely related OTU 'denovo124'. Differences characteristics for the latter are shown by nucleotides outside the structure and are linked by dotted lines. Top of helix II and helix IV (encircled) represent one of expansion segments, whose length is not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, this part of helix II and helix IV characteristic for OTU 'denovo124' is shown outside the structure and is linked by dotted lines. No CBC was found between *Lobosphaera incisa* SAG 2466 and OTU 'denovo124' suggesting that OTU 'denovo124' can be assigned to species *Lobosphaera incisa*. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

	10	20	30	40	50	60
Cd. nivalis DL07	GGUUAAUUCUCGCCCA	UCAUCUUCCO	CUGUGUUGAU	GAUCUGGGCGG	AUCUGGCUUU	CUCA
P27 GU117577.1	(((((((()	UCAUCUUCCO	UGUGUUGAU	GAUCUGGGCGG	AUCUGGCUUU	CUCA
denovo20	((((((((()	UCAUCUUCCO	UGUGUUGAU	GAUCUGGGCGG	AUCUGGCUUU	CUCA
H14 KX063729.1	(((((((()))))))))))))))))))))))))	UCAUCUUCCO	UGUGUUGAU	GAUCUGGGCGG	GAUCUGGCUUU	CUCA
denovo72	GGUUAAUUCUCGCCCA	UCAUCUUCCO	UGUGUUGAU	GAUCUGGGCGG	AUCUGGCUUU	CUCA
	70 .	80	90	100	110	120
Cd. nivalis DL07	GCUCUCACGA	G <mark>CU</mark> GGG <mark>U</mark>	JUGCUGAAGU	ACAGAGGUUAA	UGCAUGAACC	CGUU
P27 GU117577.1	GC - U - CUCACG - A (((())))	G <mark>CU</mark> GGG <mark>U</mark>	JUGCUGAAGU	ACAGAGGUUAA	UGCAUGGACC	CGUU
denovo20	GC - U - CUCACG - A (((()))	GCUGGGU	JUGCUGAAGU	CAGAGGUUAA	UGCAUGAACC	GUU
H14 KX063729.1	GUUGUUCCUCACGGAA	GAACUGGGU	JUGCUGAAGU	ACAGAGGUUAA	UGCAUGGACC	GUU
denovo72	GCUGUUCCUCACGGAA	GAGCUGGGU	JUGUUGAAGU	ACAGAGGUUAA	UGCAUGGACC	CGUA
	130 .	140	150 	160 .	170 .	180
Cd. nivalis DL07	AAGGGCUGCAGCUGGU	UAGGCAGUG/	AUGCUAUCGAU	JUAGCUGUUGG	SUCUGGGAACU	G <mark>C</mark> GU
P27 GU117577.1	AAGGGCUGCAGCUGGU	UAGGCAGUG	AUGCUAUCGAU	JUAGCUGUUGG		
))
denovo20	AAGGGCUGCAGCUGGU	UAGGCAGUG/	AUGCUAUCGAU	JUAGCUGUUGG		G <mark>C</mark> GU
denovo20 H14 KX063729.1	AAGGGCUGCAGCUGGU	UAGGCAGUGA (. ((((UAGGCAGUGA	AUGCUAUCGAU	JUAGCUGUUGG	UCUGGGAACU)))))))) UCUGGGAACU	GCGU)) GCGU
	AAGGGCUGCAGCUGGU (((((((((((((AAGGGCUGCAGCUGGU ((((((((((() AAGGGCUGCAGCUGGU	UAGGCAGUG2 ((.(((())UAGGCAGUG2 ((.(((())UAGGCAGUG2	AUGCUAUCGAU)))))) AUGCUAUCGAU))))))) AUGCUAUCGAU	JUAGCUGUUGG	UCUGGGAACU))))))))))))))))))))))))))))))))))))	GCGU)) GCGU)) GCGU
H14 KX063729.1	AAGGGCUGCAGCUGGU ((((((((((((AAGGGCUGCAGCUGGU ((((((((() AAGGGCUGCAGCUGGU ((((((((())	UAGGCAGUG2 ((. (((() UAGGCAGUG2 ((. (((() UAGGCAGUG2 ((. (((()	AUGCUAUCGAU .)))))) AUGCUAUCGAU .)))))) AUGCUAUCGAU .))))))	JUAGCUGUUGG	UCUGGGAACU))))))))))))))))))))))))))))))))))))	GCGU)) GCGU)) GCGU
H14 KX063729.1	AAGGGCUGCAGCUGGU (((((((((((((AAGGGCUGCAGCUGGU ((((((((((() AAGGGCUGCAGCUGGU	UAGCAGUG/ ((.(((() UAGCAGUG/ ((.(((() UAGCAGUG/ ((.(((() 200	AUGCUAUCGAI .))))))) AUGCUAUCGAI .))))))) AUGCUAUCGAI .))))))) 210	JUAGCUGUUGG	UCUGGGAACU))))))))))))))))))))))))))))))))))))	GCGU)) GCGU)) GCGU
H14 KX063729.1	AAGGGCUGCAGCUGGU (((((((((((((((((((((((((((((((((((UAGCAGUG/ ((.(((() UAGCAGUG/ ((.(((() UAGCAGUG/ ((.(((() 200)	AUGCUAUCGAI .))))))) AUGCUAUCGAI .)))))) AUGCUAUCGAI .)))))) 210 JAUUUCCU	JUAGCUGUUGG	UCUGGGAACU))))))))))))))))))))))))))))))))))))	GCGU)) GCGU)) GCGU
H14 KX063729.1 denovo72	AAGGGCUGCAGCUGGC (((((((((((((((((((((((((((((((((((UAGCAGUG/ ((.(((UAGCAGUG/ ((.(((UAGCAGUG/ () AAACUJAAU)))	AUGCUAUCGAI .))))))) AUGCUAUCGAI .))))))) AUGCUAUCGAI .)))))) 210 	JUAGCUGUUGG	UCUGGGAACU))))))))))))))))))))))))))))))))))))	GCGU)) GCGU)) GCGU
H14 KX063729.1 denovo72 <i>Cd. nivalis</i> DL07	AAGGGCUGCAGCUGGU (((((((((((((((((((((((((((((((((((UAGCAGUG/ ((.((((UAGCAGUG/ ((.((((UAGCAGUG/ ((.((((200))) SAAACUUAAU())) GAAACUUAAU())	AUGCUAUCGAI .))))))) AUGCUAUCGAI .))))))) AUGCUAUCGAI .))))))) 210] JAUUUCCU) JAUUUCCU) JAUUUCCU	JUAGCUGUUGG	UCUGGGAACU))))))))))))))))))))))))))))))))))))	GCGU)) GCGU)) GCGU
H14 KX063729.1 denovo72 <i>Cd. nivalis</i> DL07 P27 GU117577.1	AAGGGCUGCAGCUGGC (((((((((((((AAGGCUGCAGCUGGU ((((((((((() AAGGCUGCAGCUGGU (((((((((() 190 	UAGCAGUG/ ((.(((UUAGCAGUG/ (.((((200))) AAACUUAAU))) AAACUUAAU)) AAACUUAAU	AUGCUAUCGAI .)))))) AUGCUAUCGAI .))))))) AUGCUAUCGAI .))))))) 210) JAUUUCCU) JAUUUCCU) JAUUUCCU) JAUUUCCU	JUAGCUGUUGG	UCUGGGAACU))))))))))))))))))))))))))))))))))))	GCGU)) GCGU)) GCGU

Fig. S10. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Chlamydomonas nivalis* DL07 (accession number MF803749.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. *Chlamydomonas nivalis* P27 (accession number GU117577.1) picked up by Qiime differs from *Chlamydomonas nivalis* DL07 by single nucleotide position in a single strand region in helix III. Note that the sequence of 'denovo20' is identical with the reference sequence of DL07. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

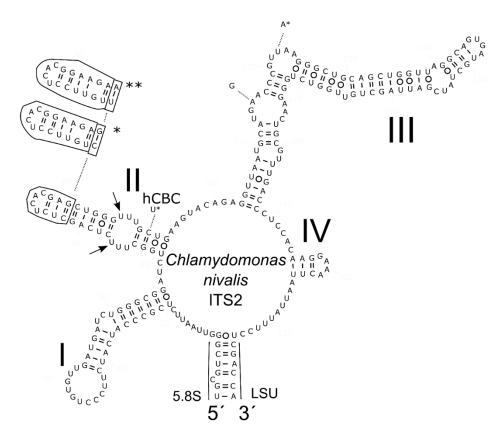


Fig. S11. Comparison of the secondary structure of ITS2 rDNA transcripts between Chlamydomonas nivalis DL07 (accession number MF803749.1), Chlamydomonas nivalis P27 (accession number GU117577.1), Chlamydomonas clone H14 (accession number KX063729.1), and the closely related OTU 'denovo20' and OTU 'denovo72'. OTU 'denovo20' was 100% identical with the reference sequence of Chlamydomonas nivalis DL07. Differences characteristic for Chlamydomonas nivalis P27, OTU denovo72 and clone H14 are shown by nucleotides outside the structure and are linked by dotted lines. Single nucleotide difference in helix III was shared in all three specimens (i.e., 'G' instead of 'A'). One asterisk means that the difference was detected only in OTU 'denovo72', and double asterisks imply that the difference was detected in clone H14. Top part of helix I (encircled) represent an expansion segments, whose length are not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, this part of helix I characteristic for OTU 'denovo72' and clone H14 are shown outside the structure and are linked by dotted lines. There was no CBC between 'denovo72' and reference sequence DL07, the sequence identity was 93%. Interestingly, ITS2 of 'denovo72' has higher sequence similarity of 98% with uncultured Chlamydomonas clone H14 (accession number KX063729.1) but there is one CBC in helix II between 'denovo72' and clone H14 (in the 13th nucleotide in helix II). A CBC at the same position in the structure is present also when reference sequence DL07 and clone H14 are compared. Single hemi-CBC (hCBC) in the basal part of helix I was found when compared Chlamydomonas nivalis DL07 and OTU 'denovo72'. Absence of any CBC in the helix III among all these sequences suggest that all these OTUs can be assigned to the same species. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

	10 20 30 40 50 60
Cr. rostafinskii	GUUCUCAAUCACAAAC-CCC-UGUGCUUGCACA-GC-C
Cr. miwae	((((((((((-(((-
denovo254	(())))))) GGUUCUCAAUCACAACAAC-CCCAUCU-CACUCAUCACGAGUCCGUGUGUAUGC-C
denovo127	(((((((((((((((())))))))))))) GGUUCUCUCAAUCACAACAACUCCCCU-UGUACUCACUCUCACGAGUCCGUGCAUACGC-C
denovo142	(())))))))))))))))))))))))))))))))
	70 80 90 100 110 120
Cr. rostafinskii	GUGUGGAAGUCGCGGUCUCUGCAUCACCUUAAC-C-GGUAUAGUGGAGUCCGCCAAAG
Cr. miwae))))))((((.(((((((((,)))))))))))) C-GUGUGGAAGUCGCGGUCCCCUCAC-CUUGUGGAGUCCGCCAAAG
denovo254))))))(.((((.(.(.(.((())))).))))).) -UGUGUGGAAAUCGCGGUCCCUUCACUCUC-G-AGUGUGGAGUCCGCCAAAG
denovo127	-))))))(.((((.(.((.(((()))))).))))) CUGUGUGGAAGUCGCGGUCCCCUCACUGUC-A-AGUGUGGAGUCCGCCAAAG
denovo142	.))))))((((.(.(.(.()))).)).)))) C-GUGUGGAAGUCGCGGUCCCCUCAUCCUCUGUGGUGUGGAGUCCGCCAAAG))))))((((.(.(.()))).)).))))
	130 140 150 160 170 180
Cr. rostafinskii	UAUGAGUAGCGCUUUCAAGCAUUGAGA-CGGAGGCC-GCAA-GGUCCUCCUGCCUCAUUC
Cr. miwae	(((((((((((((((((((())))))))
denovo254	(((((((((((((((())))))))))))) UAUGAGUAGCGCUUUCGAGCAUUGGGUCUCGC-CCC-UUCAAAAGGCCGC-GUCCCAUU- (((((((((((((((((((())))))
denovo127	UAUGAGUAGCGUUUCAAGCAUUGGU-U-GACCUCUUCAAACGGCUC-UCCCAUU- ((((((((((((((((((
denovo142	UAUGAGUAGCGCUUUCAAGCAUUGG-U-UGGCACCCCUUCAAACGGGUGU-CUCCCAUU- ((((((((((((((((((())))))))
	190 200 210 220 230 240
Cr. rostafinskii	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC
Cr. rostafinskii Cr. miwae	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((((((())))))))))))))).). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGACCUGUUUGAUC
	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.(((((((((((((((((,,)))))))))))))))))))))))) AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGACCUGUUUGAUC ((.(((((((((((((((,))))))))))))))))))))) AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGACCUGUUUGAUC (((((((((((((((,)))))))))))))))))))))) AAAGCCACACCCGAGUAUGCAUCUCCGUAUGUUGCUCGACCUUGUGCCUUGUUCGAUC
Cr. miwae	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((()))))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGCCUUGUUUGAUC
Cr. miwae denovo254	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((())))))))))))).).)). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGACCUGUUUGAUC .(.(((((((()))))))))))))))))))) AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGACCUGUUUGAUC .(.(((((((())))))))))))))))))))) AAAGCCACACCCGAGUAUGCAUCUCCGUAUGUUGCUCGACCUUGUGUCCUUGUUCGAUC .((.(((.((((.(((())))))))))))))))))))) AAAGCCACACCCGAGUAUGCAUCUUCGCAUGUUGCUCGACCUUGUUCUCGUUCGAUC .((.(((.(((((.(((())))))))))))))))))) AAAGCCACACCCGAGUAUGCAU-U-C-UAUGUAUUGCUCGACCUUGUUUGAUC .((.(((.(((((.(((()))))))))))))))))))))
Cr. miwae denovo254 denovo127	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((((((())))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUUGGAUCUUGUUUGAUC (.(((((((((()))))))))))))). AAAGCCACACCCGAGUAUGCAUCUCCGUAUGUUGCUCGACCUUGUUCGAUC (.(((((((((()))))))))))))). AAAGCCACACCCGAGUAUGCAUCUCCGUAUGUUGCUCGACCUUGUUCGAUC ((.((.((((((()))))))))))))))))
Cr. miwae denovo254 denovo127	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.(((((((((((((()))))))))))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGGACUGUUUGAUC ((((((((((((())))))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCCUUGUGUCGACCUUGUGCCUUGUUCGAUC (((((((((((((())))))))))))))))). AAAGCCACACCCGAGUAUGCAUCUCCGUAUGUUGCUCGACCUUGUGCCCUUGCUUCGAUC .(((.((((((((((((()))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((((((())))))))))))).).))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACCUGUUUGAUC ((((((((((((((()))))))))))))).)))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGCCUUGUUCGAUC (((((((((((((((()))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((((((()))))))))))))))).)).))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGGACUAGUUUGAUC (.((((((((((((())))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.(((((((((()))))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC ((.(((((((())))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGCCUUGUUCGAUC (((((((((((((((()))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.(((((((((((()))))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGACCUGUUUGAUC ((.(((((((()))))))))))))))). AAAGCCACACCCGAGUAUGCAUCUUCCGUAUGUUGCUCGACCUUGUGCCUUGUUCGAUC ((.((((((((((((()))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254 denovo127	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.(((((((((((()))))))))))))).)).)).)). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGGACCUGUUUGAUC .(.((((.((((((((((()))))))))))))).)))).)). AAAGCCACACCCGAGUAUGCAUCUUCCGUAUGUUGCUCGACCUUGUGCCUUGUUCGAUC .(((((.(((((((((((((()))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254 denovo127	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((((((()))))))))))))))).)).))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGUCUGUUUGAUC ((((((((((((((())))))))))))))))).))))). AAAGCCACACCCGAGUAUGCAUCUUCCGUAUGUUGCUCGACCUUGUUGCAUC (((((.(((((((((((((((()))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254 denovo127 denovo142	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((((((()))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii	AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.(((((((((((())))))))))))))).))))). AAAGCCACACCCGAGUAUGCAUCUUCGUAUGUUGCUCGACCUUGUGUCGUUUGAUC (.((((((((((((((((())))))))))))))))))
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae	<pre>AA-GCCGCACCCGAGUAUGCAUCUUCGUAUGUUGCUCGAUCUUGUGACUAGUUUGAUC .(-(.((((((((((((()))))))))).))))))))</pre>
Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254 denovo127 denovo142 Cr. rostafinskii Cr. miwae denovo254	<pre> </pre>

Fig. S12. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Chloromonas rostafinskii* CCCryo 010-99 (accession number

HQ404863.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. Further search in NCBI revealed that a better hit in term of higher sequence identity of 86-90% for these OTUs was represented by *Chloromonas miwae* NIES-2379 (accession number LC012762.1). As a contrast ITS2 of *Chloromonas rostafinskii* had sequence similarity with these three OTUs between 77-85% only. Compensatory base changes (CBC) in comparison with the reference sequence of *Chloromonas miwae* were found in helix III in OTU 'denovo254' (three CBCs), OTU 'denovo127' (two CBCs) and OTU 'denovo142' (four CBCs). Thus, three OTUs represent probably independent taxa. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

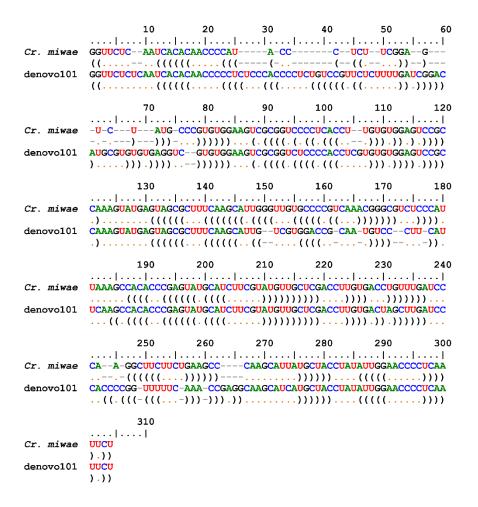


Fig. S13. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Chloromonas miwae* NIES-2379 (accession number LC012762.1) and OTU 'denovo101'. No blast hit was initially found against custom download database using Qiime. Further manual search in NCBI revealed 84% sequence similarity shared with *Chloromonas miwae* NIES-2379. Three compensatory base changes (CBCs) in comparison with the reference sequence of *Chloromonas miwae* were found in helix III, two out of the most conserved part of the structure, i.e., in the top close to the 5' end of III helix. Thus OTU 'denovo101' represents likely an independent taxon. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

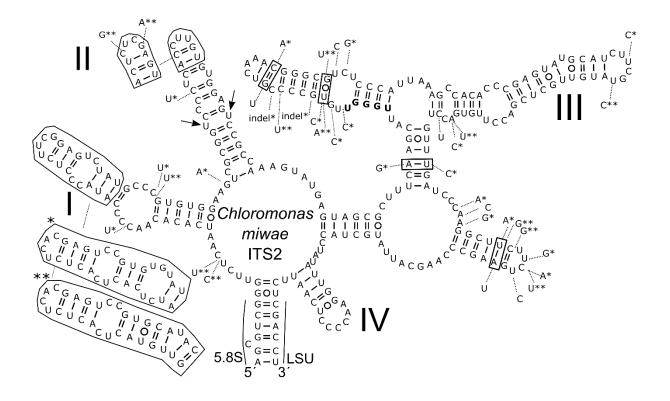


Fig. S14. Comparison of the secondary structure of ITS2 rDNA transcripts between *Chloromonas miwae* NIES-2379 (accession number LC012762) and the closely related OTU 'denovo254' and OTU 'denovo127'. Differences characteristic for both OTUs are shown by nucleotides outside the structure and are linked by dotted lines. One asterisk means that the difference was detected only in OTU 'denovo254', and double asterisks imply that the difference were detected in OTU 'denovo127'. Middle and top part of helix I and top part of helix II (encircled) represent expansion segments, whose length are not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, these parts of helix I and II characteristic for OTU 'denovo127' are shown outside the structure and are linked by dotted lines. CBSs between ITS2 sequences of *Chloromonas miwae* NIES-2379, OTU 'denovo254' and OTU 'denovo127' are indicated by rectangles in the helix III, one out of these CBC is located in the most conserved part of helix III suggesting that both OTUs represent probably two independent species. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

<i>Scotiella cryophila</i> denovo63 denovo44	10 GGUUCCCUGUCUCAACCUC (((((((((() GGUUCCCUGUCUCAACCUC ((((((((() GGUUCCCUGUCUCAACCUC (((((((()))))))))))))))))))))))))	CAACAUUUAUUUAAUU (((((CAACAUUUAUUUAAUU (((((CAACAUUUAUUUAAUU	AUAAUUGUCAGA)))),)),))),)) AUAAUUGUCAGA)))),))),))),)) AUAAUUGUCAGA	AGUGUCUUGAGG) .)))))) AGUGUCUUGAGG) .)))))) AGUGUCUUGAGG	GAC GAC GAC
<i>Scotiella cryophila</i> denovo63 denovo44	70 CGAUGGCAGUCUGGGCAUU (((((.(((CGAUGGCAGUCUGGGCAUU (((((CGAUGGCAGUCUGGGCAUU (((((UAUUUGCACAGUCUGC))).))))))) UAUUUGCACAGUCUGC))).))))))))) UAUUUGCACAGUCUGC	CCAAAUGACAUG)(CCAAAUGACAUG)(CCAAAUGACAUG	SUGGUGCUUCCU (((((((JUG (((JUG (((JUG
<i>Scotiella cryophila</i> denovo63 denovo44	130 1 CGUGGACAGGAUUGUUUAA ((((((((((((((((((((((((((((((((((((GUACAGCCCUGUUCAA)))).)))))) GUACAGCCCUGUUCAA)))).)))))) GUACAGCCCUGUUCAA	CCUAAGCCGCAC	CUCGAGUAUUCA CUCGAGUAUUCA CUCGAGUAUUCA CUCGAGUAUUCA	AUC ((. AUC ((. AUC
<i>Scotiella cryophila</i> denovo63 denovo44	190 2 UUGUAUGUCUCUCGA-CCU	CGACGUCUUGCGCAAU)))))))))) CGACGUCUUGCGCAAU)))))))))) -GCCGGUUUGGGCAAU	GAACAUGCUUCC)).(((((GAACAUGCUUCC)).(((((GAACGUC-	GUGGCAGAAUU ((((((, (((, , GGUGGCAGAAUU (((((, ((), . UGGU-GUGCU	JUA JUA JUC
<i>Scotiella cryophila</i> denovo63 denovo44	250 2 UUCGGUCGCCGUCUC))).))))))) UUCGGUCGCCGUCUC))).))))))) AAACCAC-ACCAG)))-)))))	AUGCAACCCUCCACAC))))))) AUGCAACCCUCCACAC))))))) AUGCAACCCUCCACAC))))))) ACCCUCCCCUAU	UUCAUUGGA UCAUUGGA ((((UCAAUCCUUGGA	AACCCAACACU AAACCCAACACU AAACCCAACACU	UCU)) JUCU))

Fig. S15. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Scotiella cryophila* K-1 (accession number MG253843.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. Note that the sequence of 'denovo63' is identical with the reference sequence. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

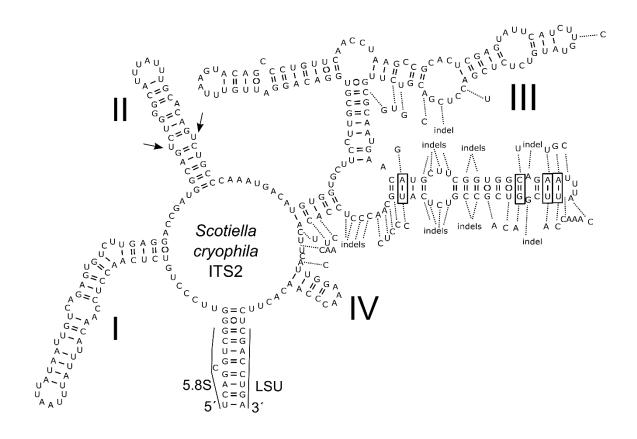


Fig. S16. Comparison of the secondary structure of ITS2 rDNA transcripts between *Scotiella cryophila* K-1 (accession number MG253843.1) and the closely related OTU 'denovo63' and OTU 'denovo44'. OTU 'denovo63' was 100% identical with reference sequence. Differences characteristic for the OTU 'denovo44' are shown by nucleotides outside the structure and are linked by dotted lines. Each out of four compensatory base changes between ITS2 sequences of *Scotiella cryophila* K-1 and OTU 'denovo44' is indicated by a rectangle in the helix III and are found outside the most conserved part of helix III (the most conserved is close to the 5'end of III helix), therefore, 'denovo44' can be assigned to *Scotiella cryophila*. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

		2
Stichococcus sp.	GGCUCACCCCUCGCCCCUCCCCUUUCAUUUGCAAAGGAGAAC-GG-GCGGACGUGGCC ((((((((())))))))))))))))	
denovo93	GGCUCACCCCUCGCCCCUCCCCUUUCAUUUGCAAAGGAGAACGG-GCGGACGUGGCC (((((((((((.(((()))))))))	
denovo109	GGCUCACCCCUCGCCCCUCCC-CUUUUCAUUUCGGAAAG-GGUUGGGAGCGGACGUGGCC ((((((((((((-(((((()))))))))	
	70 80 90 100 110 12	2
Stichococcus sp.	GUCUCGCUGCUGCUGUUAGUUUU-UAAUUAAUCGGCGGCGGGUCGGCUGAAGUGCAGGGCUCG (.((((((((((((((((, -, -))))))))))))))))))	
denovo93	GUCUCGCUGCUGUUAGUUUU-UAAUUAAUCGGCGGCGGGGGCGGGCUGAAGUGCAGGGCUCG	
denovo109	(.(((((((((((((((((())))))))))))))))	
	130 140 150 160 170 180)
Stichococcus sp.	CGGCGCGACUCUCUCAAAAGAGCGCCAGCAUGGUAGGUGGCUUCACGCUGCAUGCCGCUG (((((.(((((((((((((((((((((((((((())))))	
denovo93	CGGCGCGACUCUCUCAAAAGAGCGCCAGCAUGGUAGGUGGCUUCACGCUGCAUGCCGCUG ((((.(((((((((((((((((((((((((((()))))))	
denovo109	CGGCGCGACUCUCUCAAAAAGAGCGCCAGCAUGGUAGGUGGCUUCACGCUGCAUGCCGCUG (((((.(((((((((((((((((((((((((((((((
	190 200 210 220 230 240)
Stichococcus sp.	UCGUUCGACUGCAGAGGGCUCAAAUGCCGCUCGGCCAUAAGCAGGAAU-UUUUUCUGGCG	
denovo93	.))))))))))))))))))))))))))))))	
denovo109	.))))))))))))))))))))))))))))	
	250 260 270	
Stichococcus sp.	CCUUCGGGCAAAAGAAAA-UUCAAACCUUC	
denovo93	(())))))))))) CCUUCGGGCAAAAGAAAA-UUCAAACCUUC	
denovo109	(())))))))))) UGGAAACAAAAGAAAAAUUCAAACCUUC	
	(())))))))))	

Fig. S17. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Stichococcus* sp. SAG 2482 (accession number KX094848.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. Note that the sequence of OTU 'denovo93' is identical with the reference sequence. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

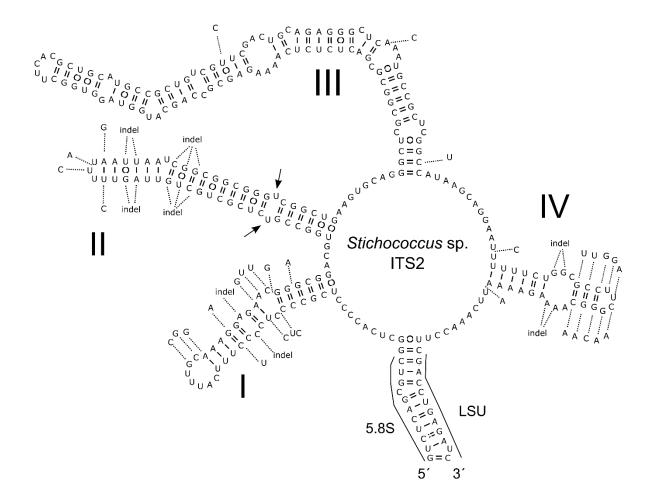


Fig. S18. Comparison of the secondary structure of ITS2 rDNA transcripts between *Stichococcus* sp. (accession number KX094848.1) and the closely related OTU 'denovo93' and OTU 'denovo109'. OTU 'denovo93' was 100% identical with reference sequence. Differences characteristic for the OTU 'denovo109' are shown by nucleotides outside the structure and are linked by dotted lines. For OTU 'denovo109' vs. reference species – six pairs of nucleotide differences at both side of structures keeping pairing were found in variable parts of the structure (the middle part of helix I, the terminal part of helix II, helix IV, i.e. no true CBCs). However, one hemi-CBC (a change at one side of the structure in the conserved part) was found in the conserved bottom part of helix III (this site was reported to be conserved across all 56 ITS2 sequences of *Stichococcus* spp. analyzed in study of (HODAČ et al. 2016). (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

	10	20	30	40	50	60
Desmococcus endolithicus	GCUCACCCCUCGCCCC	UCCCCGAC-	CCACCCCGCAA	GGGG <mark>CCU</mark> GGG	CCGGGGCCUC	GGAC
denovo181	((((.((GCUCACCCCUCG-CCC	CACCCGACA	C-CCCCGAAA	GGGAGGG	UCGGGCCCC	GG-C
denovo13	((((-((GCUCACCCCUCG-CC					
	((((-(((((((((((()))))))))))))-)
	70 	80 I I	90	100	110	120
Desmococcus endolithicus	GGAUGUGGCCGUCUC	GCUGCUUGA	AACACAGCGCC	GGGUCGGCUG	AAGCACAGGO	CUUG
denovo181	GGACGUGGUCGUCUC	GGCACUU-GC	AACACAGAGCC	GGGUCGACUG	AAGCACAGGO	CUUG
denovo13))(((((((((((((
))(((((((((((()).)))))).))))))))	(((((
	130	140	150	160	170	180
Desmococcus endolithicus	CUGCCGGUCACCCCC	JC-UCCCA	GCACGGGGGCGC	CAGCUUGGUA	GGUGGCUUU	UAAG
denovo181	CUGCCGGACACCCCC	JUUCCG-UGC	AAA-GGGGCGC	CAGCUUGGUA	GGUGGCU	JUG
denovo13		JU-UCGCUGC	AAA-GGGGCGC	CAGCUUGGUA	GGUGGCU	JUU-G
	(((((((– ((((((((((((((((((. ((((()
	190 	200 	210 .	220	230	240
Desmococcus endolithicus	CUGCAUGCCGCUGUC	G-CGCC-GAC	CGCAUGGCGAG	CGAAAGAG	GCCG-G-UCC	CAGC
denovo181	CCGCAUGCCGCUGUC	G-UUCCCG-C	CGCUUCGG	UUUGAAAGAG	UCCGUGCC	AGC
denovo13	CCGCAUGCCGCUGUC	GUCUCC-G-C	CGCUUCGA	UU-GAAAGAG	UCCGUGCC	AGC
))))-))))))))))).)))))))))))))).).))))
	250 	260 	270 .	280 .	290	300
Desmococcus endolithicus	GCGGCCAAGCAGGAA					
denovo181	GCGGCCAAGCAGGAA	ACCUGCGG-C	GAUGCG-GCCU	CCGAAAGGGG	GCCGCGGAC	ACCGC
denovo13	GCGGCCAAGCAGGAA	ACCUGCGGGA	AA-GG- <mark>C</mark> GAG <mark>U</mark>	CCACCAGGGC	UCCCC-GAC-	CCGC
).))))		•••••••••••••••••••••••••••••••••••••••	(())))))•))-•)-	-,,,,
	310 .					
Desmococcus endolithicus	ACACACCCUUU					
denovo181	ACCAACCCUUU					
denovo13	ACCAACCCUUU					
	,					

Fig. S19. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Desmococcus endolithicus* SAG 25.92 (accession number KX094830.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

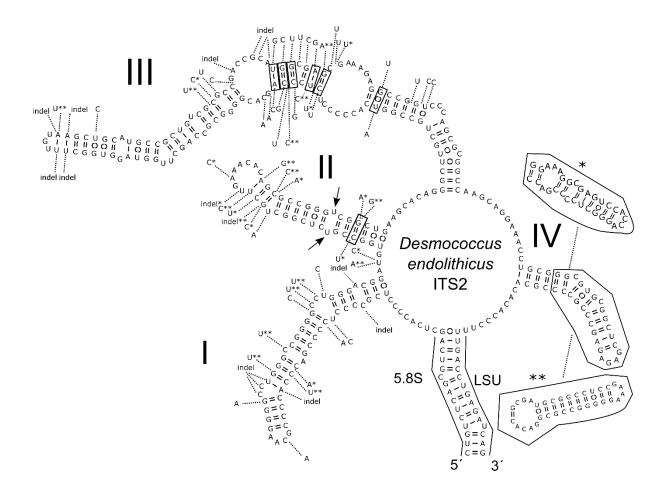


Fig. S20. Comparison of the secondary structure of ITS2 rDNA transcripts between Desmococcus endolithicus SAG 25.92 (accession number KX094830.1) and the closely related OTU 'denovo181' and OTU 'denovo13'. Differences characteristic for these OTUs are shown by nucleotides outside the structure and are linked by dotted lines. One asterisk means that the difference was detected only in OTU 'denovo181', and double asterisks imply that the difference were detected in OTU 'denovo13'; middle and top part of helix IV (encircled) represent an expansion segment, whose length is not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, this part of helix IV characteristic for both OTUs is shown outside the structure and is linked by dotted lines. Compensatory base changes between ITS2 sequences of Desmococcus endolithicus SAG 25.92 and OTU 'denovo181', and between Desmococcus endolithicus SAG 25.92 and OTU 'denovo13' are indicated by rectangles in the helix II and in the helix III. OTUdenovo181 and OTUdenovo13 had five and four CBCs, respectively, in conserved parts of the ITS2 secondary structure in comparison with the reference sequence. Moreover, ITS2 sequence similarities between Desmococcus endolithicus SAG 25.92 vs. OTU 'denovo181' and OTU 'denovo13' were only 82% and 84%, respectively (e.g. below the suggested identity threshold <89%). (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

<i>Prasiola delicata</i> denovo69	10 . GCUCACCCCUCGC ((((GCUCACCCCUCGCCCC) (((((((.	-CCGGACCC -((((,(() ACCCGACCC	CGCCCGCCCU (((((CUCCCGA	UCGAGGCAGU	CGG-GGCCCG()))-))))) CGGCGGCCGG(G) G <mark>UCC</mark> G
<i>Prasiola delicata</i> denovo69	70 	 UCGGCACCC (((((.(() UCGGU-GCC	UGCGACACGG	GAGCCGGGUC).))))))))))) C-GCCGGGUC	GGCUGAAGCAG	CAGGG ((CAGGG
<i>Prasiola delicata</i> denovo69	130 . CUAGCCGACGGUCG ((.((((((((CUAGCUGCCGGACA) (.(((((((((CCCCGCCCC (. (((((((CCCCUUUCG	 CCG-GU-GGU (((-(((. CCGCAUAGG-	GCGUCGCCAG ((((((,(() G-G-CGCCAG	CUUGGUAGGU((((((.((CUUGGUAGGU(GG <mark>UCC</mark> ((((. GG- <mark>CC</mark>
<i>Prasiola delicata</i> denovo69	190 . CCGAGACCGCAUGCCG)))))).)))) UU-AG-CCGCAUGCCG)-)))).))))	 CUGUCGG))).)))) CUGUCGUUU	 CGCC-CCCG -))))-)))) CCCGCCGCUCC	 GGAGGUGC)).)).)) GAUC-GA-AC	GGAG <mark>CCCGUU</mark>)).))))))))))) GGA- <mark>UCC</mark> G-GC	CGU-G))) CCCAG
<i>Prasiola delicata</i> denovo69	250 . CACGGCCAAAACAGGA))))) CACGGCCAAG-CAGGA)))))	AGCCGGGGGG (((((AAACGUGCG	 GGAGCCC- (((((GG <mark>UGCCUCCG</mark>	-CUCCCCCCC -)))))))))) GCGCCCGCAC	UUUCGUUU) ACAACC-C	

Fig. S21. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Prasiola delicata* isolate I34S (accession number MF801375.1) and OTU 'denovo69'. No blast hit was initially found against custom download database using Qiime. Further manual search in NCBI revealed 82% sequence similarity shared with *Prasiola delicata* isolate I34S. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

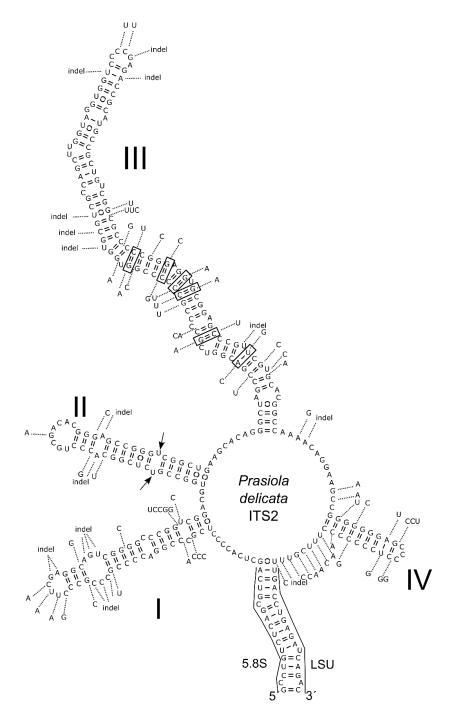


Fig. S22. Comparison of the secondary structure of ITS2 rDNA transcripts between *Prasiola delicata* isolate I34S (accession number MF801375.1) and OTU 'denovo69'. Differences characteristic for this OTU are shown by nucleotides outside the structure and are linked by dotted lines. Compensatory base changes in conserved parts of the structure are indicated by rectangles. OTU 'denovo69' had six CBCs in helix III of the ITS2 secondary structure in comparison with the reference sequence. Moreover, ITS2 sequence similarities between *Prasiola delicata* isolate I34S and OTU 'denovo69' were only 82% (i.e. below the suggested identity threshold <89%). (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

Chl. engadinensis denovo180	10 GUUGACCCUUUCACCC (.((((((((GUUGACCCCUUCAUUC (.(((((((()	GCCUCCCUCA	CAGGAGAAGG))))))) CGGGAGGAGGAGG	GUGGAUCUGG)))))(((AUGGACCUGG	CAGUCCCGCU (((.((((() CAGUCCCGCU	GGA (GGC
Chl. engadinensis denovo180	70 AACAGCGGGUCUGCUGC))))))))))) GACAGCGGGUCUGCUGC))))))))))))	AGACCAGACG		UCGCUUAUGC		GGA ((GGA
Chl. engadinensis denovol80	130 UUGGUAGGUUCCCCUA- (.((((.(((UUGGUAGGUU-CCCUUU (.((((GCAGGGUACA	. .UGCCCAUCGA)))).))))))))))))))))))	GUGGCUGGGC))))))),))),))),))),))),)))))))))))	. CUGACAACUG .)))))) UCGACACCUG	CAC)))) CGC
<i>Chl. engadinensis</i> denovo180	190 GUUUCCAACCAC-GAG-)))(((- GUUCACAACCACAGAGC)))((((.	GGCAGUGGGG ((((((()))))) GGUGGAGGUG	UACUCUCCCA		UUCUCAGUU)))) CUCUCAGUU	

Fig. S23. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Chlorocloster engadinensis* SAG 812-1 (accession number FM946011.1) and OTU 'denovo180'. No blast hit was initially found against custom download database using Qiime. Further manual search in NCBI revealed 84% sequence similarity shared with *Chlorocloster engadinensis* SAG 812-1. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

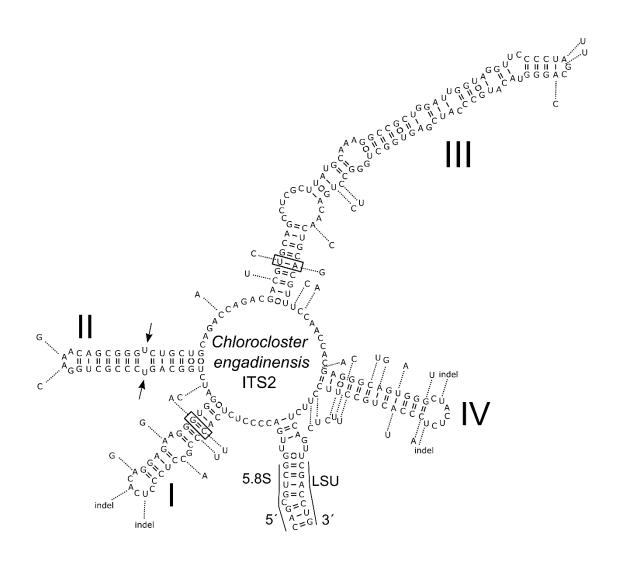


Fig. S24. Comparison of the secondary structure of ITS2 rDNA transcripts between *Chlorocloster engadinensis* SAG 812-1 (accession number FM946011.1) and OTU 'denovo180'. Differences characteristic for this OTU are shown by nucleotides outside the structure and are linked by dotted lines. Compensatory base changes in conserved parts of the structure are indicated by rectangles. OTU 'denovo180' had one CBC in basal part of helix I and one CBC in basal part of helix III in comparison with the reference sequence. Moreover, ITS2 sequence similarities between OTU 'denovo180' and its closest hit in NCBI, *Chlorocloster engadinensis* SAG 812-1, were only 84% (i.e. below the suggested identity threshold <89%). (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

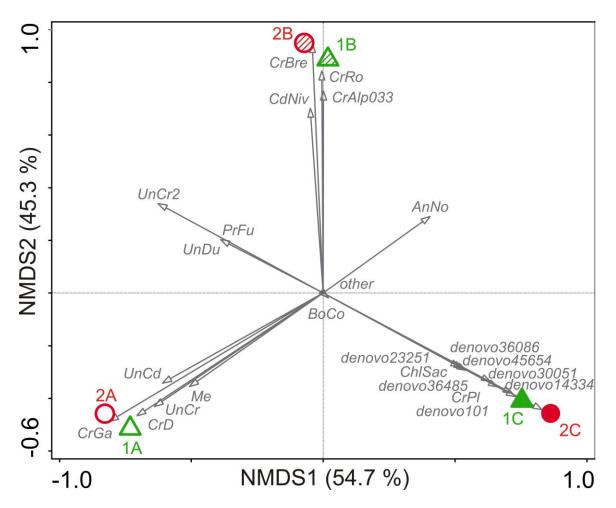


Fig. S25. Nonmetric multidimensional scaling (NMDS) ordination describing the differences in taxonomic composition of the two samples (sample 1 – green, sample 2 – red) based on 18S rDNA data analysed using the three strategies of taxonomic assignment (A, B, C – for details, see the text). Values within parentheses show % of variance explained by each axis. Source of the data for NMDS ordination: Table 5. Acronyms:

AnNo	Ancylonema nordenskioeldii AF514397.2
BoCo	Botrydiopsis constricta AJ579339.1
CdNiv	Chlamydomonas nivalis DL07 MF803748
CrAlp033	Chloromonas cf. alpina CCCryo 033-99 HQ404865.1
CrBre	Chloromonas brevispina K-2 MG791867
CrD	Chloromonas sp. D-CU581C AF517086.1
CrGa	Chloromonas sp. Gassan-A LC012753.1
CrPl	Chloromonas platystigma strain CCCryo 020-99 AF514401.1
CrRo	Chloromonas cf. rostafinskii CCCryo 025-99 AF514402.1
ChlSac	Chloroidium saccharophilum isolate HST10K KX024691.1
Me	Mesotaenium sp. AG-2009-1 FM992335.1
PrFu	Prasiola furfuracea AF189073.1
UnCd	Uncultured Chlamydomonadaceae AB902971.1
UnCr	Uncultured Chloromonas AB903008.1
UnCr2	Uncultured Chloromonas AB902984.1
UnDu	Uncultured Dunaliellaceae EF023287.1

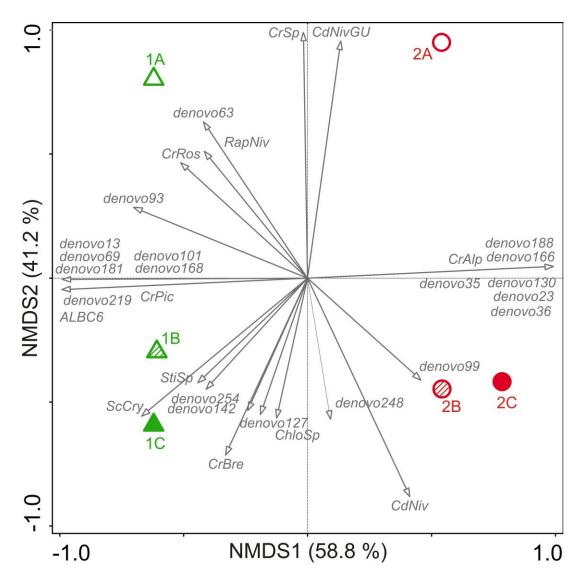


Fig. S26. Nonmetric multidimensional scaling (NMDS) ordination describing the differences in taxonomic composition of the two samples (sample 1 – green, sample 2 – red) based on ITS2 data analysed using the three strategies of taxonomic assignment (A, B, C – for details, see the text). Values within parentheses show % of variance explained by each axis. Source of the data for NMDS ordination: Table S7.

Acronyms:

ALBC6	Uncultured Chlorophyte clone ALBC6 JX435348
CdNiv	Chlamydomonas nivalis DL07 MF803749
CdNivGU	Chlamydomonas nivalis GU117577
CrAlp	Chloromonas alpina CCCryo032-99 HQ404864
CrBre	Chloromonas brevispina K-2 MG791868
CrPic	Chloromonas pichinchae CCCryo 2616-06 HQ404889
CrRos	Chloromonas rostafinskii HQ404863
CrSp	Chloromonas sp. CCCryo 289-06 HQ404893
ChloSp	uncultured Chlorella sp.5 AB90311
RapNiv	Raphidonema nivale AJ431676
ScCry	Scotiella cryophila K-1 MG253843
StiSp	Stichococcus sp. SAG 2482 KX094848

Table S1. List of primers used for amplification and Sanger sequencing reactions of 18S rDNA and ITS2 rDNA markers; (F) forward; (R) reverse.

Primer	Marker	Direction	Sequence	Reference
P2	18S	F	CTGGTTGATTCTGCCAGT	(MOON-VAN DER STAAY et al. 2000)
P4	18S	R	TGATCCTTCYGCAGGTTCAC	(MOON-VAN DER STAAY et al. 2000)
FA	18S	F	AACCTGGTTGATCCTGCCAGT	(MATSUZAKI et al. 2015)
RD	18S	R	GCTGGCACCAGACTTGCCCTC	(MATSUZAKI et al. 2015)
FC	18S	F	GGGAGGTAGTGACAATAAATA	(MATSUZAKI et al. 2015)
RF	18S	R	CCCGTGTTGAGTCAAATTAAG	(MATSUZAKI et al. 2015)
FE	18S	F	GGGAGTATGGTCGCAAGGCTG	(MATSUZAKI et al. 2015)
RB	18S	R	TGATCCTTCTGCAGGTTCACCTAC	(MATSUZAKI et al. 2015)
AL1500af	ITS	F	GCGCGCTACACTGATGC	(HELMS et al. 2001)
LR3	ITS	R	GGTCCGTGTTTCAAGACGG	(VILGALYS & HESTER 1990)
ITS5	ITS	F	GGAAGTAAAAGTCGTAACAAGG	(WHITE et al. 1990)
ITS4	ITS	R	TCCTCCGCTTATTGATATGC	(WHITE et al. 1990)
SSU	ITS	F	CTGCGGAAGGATCATTGATTC	(Piercey-Normore & DePriest 2001)
LSU	ITS	R	AGTTCAGCGGGTGGTCTTG	(PIERCEY-NORMORE & DEPRIEST 2001)

Supplemental References

- CAISOVÁ, L.; MARIN, B. & MELKONIAN, M. (2013): A Consensus Secondary Structure of ITS2 in the Chlorophyta Identified by Phylogenetic Reconstruction. Annals of Anatomy 164: 482–496.
- HELMS, G.; FRIEDL, T.; RAMBOLD, G. & MAYRHOFER, H. (2001): Identification of photobionts from the lichen family Physiaceae using algal-specific ITS rDNA sequencing. The Lichenologist 33(1): 73–86.
- HODAČ, L.; HALLMANN, C.; SPITZER, K.; ELSTER, J.; FABHAUER, F.; BRINKMANN, N.; ... FRIEDL, T. (2016): Widespread green algae Chlorella and Stichococcus exhibit polartemperate and tropical-temperate biogeography. – FEMS Microbiology Ecology 92(8): 1–16.
- MATSUZAKI, R.; KAWAI-TOYOOKA, H.; HARA, Y. & NOZAKI, H. (2015): Revisiting the taxonomic significance of aplanozygote morphologies of two cosmopolitan snow species of the genus Chloromonas (Volvocales, Chlorophyceae). Phycologia 54(5): 491–502.
- MOON-VAN DER STAAY, S.Y.; VAN DER STAAY, G.W.M.; GUILLOU, L.; VAULOT, D.; CLAUSTRE, H. & MEDLIN, L.K. (2000): Abundance and diversity of prymnesiophytes in the picoplankton community from the equatorial Pacific Ocean inferred from 18S rDNA sequences. – Limnology and Oceanography 45(1): 98–109.
- PIERCEY-NORMORE, M.D. & DEPRIEST, P.T. (2001): Algal switching among lichen symbioses. American Jurnal of Botany 88(8): 1490–1498.
- VILGALYS, R. & HESTER, M. (1990): Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptoccocus species. – Journal of Bacteriology 172(8): 4238–4246.
- WHITE, T.J.; BRUNS, T.; LEE, S. & TAYLOR, J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. INNIS; D. H. GELFAND; J. J. SNINSKY, & T. J. WHITE (eds.): PCR Protocols. pp. 315–322, Elsevier.

Taxon	Specimen/strain designation	Accession number ITS2 rDNA
Chlamydomonas reinhardtii	CC620	U66954.2
Chlamydomonas sp.		AF033295.1
Raphidonema nivale	CCAP 470/4	AJ431676.1
Raphidonema sempervirens	CCAP 470/6	AJ431674.1
Spirogyra sp.	GRS2	KM453687.1
Klebsormidium sp.	SAG 2065	EU434032.1
Mesostigma viride		EC732140.1
Chloromonas nivalis	P24/DR4	GU117576.1
Chloromonas nivalis	CCCryo_005-99	HQ404862.1
Chloromonas pichinchae	CCCryo_261-06	HQ404889.1
Chloromonas sp.	CCCryo 207-05	HQ404883.1
Chloromonas alpina	CCCryo 033-99	HQ404865.1
Chloromonas alpina	CCCryo 032-99	HQ404864.1
Chloromonas sp.	CCCryo 289-06	HQ404893.1
Chloromonas rostafinskii	CCCryo 010-99	HQ404863.1
Chloromonas pichinchae	CCCryo 192-04	HQ404880.1
Chlamydomonas nivalis	P27/DR1	GU117577.1
Chloromonas sp.	JP13	AB902970.1
Uncultured Chlorophyta	clone ALBC6	JX435348.1

Table S2. ITS2 reference sequences downloaded from NCBI for the custom-built database

Table S3. List of reference species locally abundant in the studied Austrian alpine snowfields
and the GenBank accession numbers of encoded 18S rDNA and ITS2 rDNA markers.

	accession number	r
Taxon	18S rDNA	ITS2 rDNA
Chlamydomonas nivalis DL07	MF803748	MF803749
Scotiella cryophila K-1	MG253843	MG253843
Chloromonas brevispina K-2	MG791867	MG791868

Table S4. Algal community compositions based on the 18S rDNA data sets comprising the 50 most abundant OTUs that made up >87% of the community. The table shows the discrepancies between OTU assignments using three strategies: (A) basic version using Qiime and the publicly available Silva database, (B) extended version using Qiime and additional reference sequences of the locally abundant taxa (underlined), and (C) further extended version using the final manual verification of taxa assignments in NCBI, only allowing a 2 bp nucleotide difference (\geq 99.4% identity; sequences below this threshold were recorded as "no blast hit"). With the respective reference sequence in order to be recorded as a match. Ambiguous hits are sequences that share all the same level of similarity with an inspected OTU, and thus they cannot be assigned unambiguously. Our results reveal that the manual verification in NCBI is essential and, thus, highly recommended.

OTU ID	Sample	Sample	(A) Qiime	(B) Qiime	(C) Qiime
	1 [%]	2 [%]	+ Silva	+ Silva	+ Silva
				+ local references	+ local references
					+ manual verification
denovo14334	33.0	66.8	Chloromonas sp. Gassan-A LC012753.1	Chloromonas brevispina K-2	Ambiguous hits:
					Chloromonas brevispina K-2
					Scotiella cryophila K-1
					Chloromonas sp. TA 8 AB902996
					Chloromonas sp. Gassan-B LC012714
denovo45654	18.7	0.1	Mesotaenium sp. AG-2009-1 FM992335.1	Ancylonema nordenskioeldii AF514397.2	Ancylonema nordenskioeldii AF514397.2
denovo36485	0.9	13.6	Uncultured Chlamydomonadaceae AB902971.1	Chlamydomonas nivalis DL07	Chlamydomonas nivalis DL07
denovo40226	8.2	0	Botrydiopsis constricta AJ579339.1	Botrydiopsis constricta AJ579339.1	Botrydiopsis constricta AJ579339.1
denovo15070	4.6	1.0	Uncultured Chloromonas AB903008.1	Chloromonas cf. alpina CCCryo 033-99	Chloromonas platystigma strain CCCryo 020-99
				HQ404865.1	AF514401.1
denovo20542	4.6	0	Uncultured Dunaliellaceae EF023287.1	Uncultured Dunaliellaceae EF023287.1	Chloroidium saccharophilum isolate HST10K
					KX024691.1
denovo101	3.2	1.1	Chloromonas sp. D-CU581C AF517086.1	Chloromonas cf. rostafinskii	Ambiguous hits [:]
				CCCryo 025-99 AF514402.1	Chloromonas sp. NIES-2379 AB906350.1,
					Chloromonas rostafinskii strain CCCryo 025-99
					AF514402.1
denovo23251	3.0	< 0.1	Chloromonas sp. Gassan-A LC012753.1	Chloromonas brevispina K-2	Ambiguous hits ¹
					Chloromonas brevispina K-2
					Chloromonas sp. Hakkoda-1 LC012710.1,
					Chloromonas sp. Gassan-A LC012709.1
denovo30051	0.4	1.9	Chloromonas sp. JP15 AB902984.1	Chloromonas sp. JP15 AB902984.1	No blast hits
denovo36086	2.1	0	Prasiola furfuracea AF189073.1	Prasiola furfuracea AF189073.1	No blast hits
denovo2551	1.6	0.1	Chloromonas sp. Gassan-A LC012753.1	Chloromonas sp. TA 8 AB902996.1	Ambiguous hits:
					Scotiella cryophila K-1
					Chloromonas sp. Gassan-B LC012714
denovo44194	0.9	0.4	Chloromonas sp. D-CU581C AF517086.1	Chloromonas tughillensis UTEX_SNO_88 AB734116.1	No blast hit

denovo30674	0.1	1.0	Prototheca cutis AB470468.1	Prototheca cutis AB470468.1	No blast hit
denovo32383	1.1	0	Chloromonas tughillensis UTEX_SNO_88 AB734116.1	Chloromonas tughillensis UTEX_SNO_88 AB734116.1	Chloromonas fukushimae AB906342
denovo12840	0	1.0	Chloromonas sp. JP15 AB902984.1	Chloromonas sp. JP15 AB902984.1	Ambiguous hits: >40 sequences including <i>Chloromonas</i> sp. TA 9 AB903024.1
denovo12413	0	1.0	Chlamydomonas sp. GL1 AB902971.1	Chlamydomonas nivalis DL07	Chlamydomonas nivalis DL07
denovo14574	0	0.9	Chlamydomonas sp. GL1 AB902971.1	Chlamydomonas nivalis DL07	Chlamydomonas nivalis DL07
denovo18060	0	0.8	Chloromonas sp. GL4 AB903027.1	Chloromonas sp. GL4 AB903027.1	Uncultured eukaryote clone ALA5117773
denovo44928	0.1	0.7	Uncultured eukaryote clone TE107A KM870650.1	Uncultured eukaryote clone TE107A KM870650.1	Uncultured eukaryote clone TE107A KM870650.
denovo25214	0.8	0	Botrydiopsis callosa AJ579340.1	Botrydiopsis callosa AJ579340.1	Botrydiopsis callosa AJ579340.1
denovo18262	0	0.7	Hydrurus sp. Sva 10_3 HE820740.1	Uncultured Hydrurus HE820740.1	Hydrurus foetidus FM955256.1
denovo12567	0.1	0.5	Chloromonas sp. Gassan-A LC012753.1	Chloromonas brevispina K-2	Ambiguous hits 7 sequences including: <u>Chloromonas brevispina K-2</u> <u>Scotiella cryophila K-1</u> Chloromonas sp. Gassan-B LC012714.1 Chloromonas polyptera JQ790556.1
denovo40835	0	0.4	Chloromonas sp. Gassan-A LC012753.1	Chloromonas brevispina K-2	No blast hit
denovo40500	0.1	0.2	Chloromonas sp. Gassan-A LC012753.1	Chloromonas brevispina K-2	Ambiguous hits: <u>Chloromonas brevispina K-2</u> <u>Scotiella cryophila K-1</u> <u>Chloromonas</u> sp. Gassan-B LC012714.1
denovo46231	0	0.3	Chloromonas sp. AL4 AB903027.1	Chloromonas sp. AL4 AB903027.1	Chloromonas sp. AL4 AB903027.1
denovo48297	0	0.3	Chloromonas sp. Hakkoda-1 LC012710.1	Chloromonas brevispina K-2	No blast hit
denovo45502	0	0.3	Botrydiopsis constricta AJ579339.1	Botrydiopsis constricta AJ579339.1	Botryidiopsidaceae sp. AM695636.1
denovo24516	0.3	0	Raphidonema sempervirens AJ309939.1	Raphidonema sempervirens AF514410.2	Ambiguous hits: >40 sequences including Raphidonema sempervirens AF514410.2 Stichococcus sp. KP08194
denovo21882	0.3	0	Heterococcus fuornensis AM490821.1	Heterococcus fuornensis AM490821.1	Ambiguous hits: 6 sequences including Heterococcus chodatii AM490822.1 Heterococcus fuornensis AM490821.1 Heterococcus pleurococcoides AJ579335.1
denovo29189	0.2	0	Stichococcus bacillaris JQ315605.1	Stichococcus jenerensis DQ275461.1	Ambiguous hits 4 sequences including Stichococcus sp. SAG 2482 KP081395.1 Stichococcus sp. KP081396.1
denovo34115	0.2	0	Chloromonas sp. JP15 AB902984.1	Chloromonas sp. JP15 AB902984.1	Ambiguous hits: >40 sequences including

					Chloromonas sp. TA9 AB903024.1 Chloromonas sp. SV3 AB903022.1 Chloromonas sp. PA2 AB903020.1
denovo16044	0.2	0	Chloromonas sp. Gassan-A LC012753.1	Chloromonas sp. Hakkoda-1 LC012710.1	Ambiguous hits: <i>Chloromonas</i> sp. Hakkoda-1 LC012710.1 <i>Chloromonas</i> sp. Gassan-A LC012709.1
denovo4533	0.1	0.2	Chloromonas sp. Gassan-A LC012753.1	<u>Chloromonas brevispina K-2</u>	Ambiguous hits: 8 sequences including <u>Chloromonas brevispina WP124</u> <u>Scotiella cryophila K-1</u> <u>Chloromonas sp. Gassan B LC012714.1</u> <u>Chloromonas polyptera JQ790556.1</u>
denovo31489	0.2	0	Prasiola furfuracea AF189073.1	Stichococcus bacillaris SAG 379-1b AJ311637.1	No blast hit
denovo17414	0	0.2	Chloromonas sp. D-CU581C AF517086.1	Chloromonas nivalis CCCryo-005-99	No blast hit
denovo47016	0	0.2	Chloromonas sp. Gassan-A LC012753.1	<u>Chloromonas brevispina K-2</u>	Ambiguous hits: 8 sequences including <u>Scotiella cryophila K-1</u> Chloromonas sp. Gassan-B LC012714.1 Chloromonas polyptera JQ790556.1
denovo38221	0.2	0	Prototheca cutis AB470468.1	Prototheca cutis AB470468.1	No blast hit
denovo13893	0	0.2	Chlamydomonas sp. GL1 AB902971.1	Chlamydomonas nivalis DL07	No blast hit
denovo16269	0	0.2	uncultured Chlamydomonadaceae HW01/1 GU117574.1	Chlainomonas sp. MF803743	Chlainomonas sp. DL06 MF803743
denovo43924	0	0.2	Chloromonas sp. Gassan-A LC012753.1	Chloromonas brevispina K-2	No blast hit
denovo34049	0.2	0	Chloromonas sp. Hakkoda-1 LC012710.1	Chloromonas sp. Hakkoda LC012710.1	No blast hit
denovo24547	0.1	0	Mesotaenium sp. AG-2009-1 FM992335.1	Ancylonema nordenskioeldii AF514397.2	Ancylonema nordenskioeldii AF514397.2
denovo15255	0.1	0	uncultured Dunaliellaceae EF023287.1	uncultured Dunaliellaceae EF023287.1	No blast hit
denovo28329	0.1	0	Heterococcus fuorensis AM490821.1	Heterococcus fuorensis AM490821.1	No blast hit
denovo23345	0.1	0	Mesotaenium sp. AG-2009-1 FM992335.1	Ancylonema nordenskioeldii AF514397.2	Ancylonema nordenskioeldii AF514397.2
denovo18826	0	0.1	Raphidonema sempervirens AJ309939.1	Raphidonema sempervirens AF514410.2	Ambiguous hits: >20 sequences including Raphidonema sempervirens AF514410.2 Raphidonema nivale AB488604.1 Koliella sempervirens AM412750.1
denovo34529	0	0.1	Chlamydomonadas sp. GL1 AB902971.1	Chlamydomonas nivalis DL07	Chlamydomonas nivalis DL07
denovo9133	0	0.1	Chloromonas sp. Gassan-A LC012753.1	Chloromonas brevispina K-2	No blast hit
denovo44422	0	0.1	Hydrurus foetidus FM955256.1	Hydrurus foetidus FM955256.1	Hydrurus foetidus FM955256.1
denovo8805	0	0.1	Chloromonas sp. Gassan-A LC012753.1	Chloromonas cf. rostafinskii HQ404863	No blast hit
Other	13.7	4.9			

Table S5. Oligotype sequences of the three most abundant 18S rDNA OTUs .

OTU ID	Oligotype ID	Sequence
denovo14434	TTT	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGT
	TTC	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAAGCTCGTAGTTGGATTTCGGGTGGGT
	TCT	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGT
	CTT	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAAGCTCGTAGTTGGATTTCGGGTGGGT
denovo36485	Т	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGT
	С	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAAGCTCGTAGTTGGATTTCGGGTGGGGTTCGGTCGG
denovo45654	С	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGGTTGGTGGGGGGGG
	А	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGGTTGGTGGGGGGGG

Table S6. Output of the ITSx software. ITS2 regions were extracted from all sequences to avoid the inclusion of the highly conserved neighbouring genes (i.e., 5.8S and 28S). Inclusion of these regions in the identification process would otherwise lead to misleading results. HMMER was used to predict the origin of the sequences (e.g., Chlorophyta, Fungi) based on Hidden Markov Models.

Number of sequences in input file:	273404
Sequences detected as ITS by ITSx:	273309
On main strand:	273239
On complementary strand:	70
Sequences detected as chimeric by ITSx:	0
ITS sequences by preliminary origin	
Alveolates:	35443
Amoebozoa:	0
Bacillariophyta:	1
Brown algae:	2
Bryophytes:	0
Euglenozoa:	0
Eustigmatophytes:	0
Fungi:	47293
Green algae:	189922
Liverworts:	0
Metazoa:	11
Microsporidia:	0
Oomycetes:	1
Prymnesiophytes:	0
Raphidophytes:	0
Red algae:	0
Rhizaria:	0
Synurophyceae:	0
Tracheophyta:	636

Table S7. Algal community composition based on the ITS2 data set and comprising the 38 most abundant OTUs that made up >98% of the community. Table shows the discrepancies between OTU assignments using three strategies: (A) basic version using Qiime and a custom database downloaded from NCBI, (B) extended version using Qiime and additional reference sequences of the locally abundant taxa (underlined), and (C) further extended version using final manual verification of taxa assignments. The latter one comprised the comparison of the representative sequences of the OTUs with their respective reference sequences in terms of sequence identities (\geq 89.0% similarity required; sequences below this threshold were recorded as "no blast hit") and their secondary structures (absence of compensatory base changes (CBC) in homological positions near the 5′- apex of helix III [the most conserved region of the ITS2 secondary structure of eukaryotes] required). Our results reveal that the manual verification including secondary structure prediction and CBC search is essential, and thus, highly recommended.

OTU ID	WP79	WP99	(A) Qiime	(B) Qiime	(C) Qiime
	[%]	[%]	+ NCBI database	+ NCBI database	+ NCBI database
				+ local references	+ local references
					+ manual verification
					(sequence similarity [%], sequence cover [%])
denovo99	1.8	59.1	Chloromonas sp. CCCryo289-06 HQ404893.1	Chloromonas brevispina K-2	No blast hit
					(88%, 83%, 6 CBC when compared denovo99 and
					Chloromonas brevispina K-2 - one CBC out of it is located i
					the most conserved part of the structure, i.e., in the top close
					the 5'end of III helix, see Fig. 4)
denovo20	5.5	28.2	Chlamydomonas nivalis GU117577.1	Chlamydomonas nivalis DL07	Chlamydomonas nivalis DL07 (100%, 100%)
denovo107	39.8	< 0.1	Chloromonas sp. CCCryo289-06 HQ404893.1	Chloromonas brevispina K-2	Chloromonas brevispina K-2 (100% identical except for one
					nucleotide - instead of \hat{R} in reference sequence, there was \hat{G} ,
					100%)
denovo100	13.7	< 0.1	Chloromonas pichinchae HQ404889.1	Chloromonas pichinchae HQ404889.1	Chloromonas pichinchae HQ404889.1
					(92%, 100%, 1 CBC in helix III [outside the most conserved
					part] when compared denovo100 and Chloromonas
					pichinchae, see Figs S6, S7)
denovo63	`10.9	0.2	No blast hit	Scotiella cryophila K-1	<u>Scotiella cryophila K-1</u> (100%, 100%)
denovo142	8.2	1.7	Chloromonas rostafinskii HQ404863.1	Chloromonas rostafinskii HQ404863.1	No blast hit
					(79%, 60% - denovo142 vs. Chloromonas rostafinskii: 86%,
					77% - denovo142 vs. Chloromonas miwae LC012762.1, fou
					CBCs [one CBC out of it is located in the most conserved pa
					of the structure, i.e., in the top close to the 5'end of III helix]
denovo130					when compared denovo142 and Chloromonas miwae,
					sequence-structure alignment in Fig. S12)
	1.4	3.5	No blast hit	No blast hit	No blast hit
1 101			XY 11	XY 11 . 11.	(no significant similarity found)
denovo181	4.1	0	No blast hit	No blast hit	No blast hit
					(82%, 87%, Desmococcus endolithicus KX094830.1; five
					CBCs – one in helix II and four CBCs in helix III, see Figs
1 254	2.1	0.1			<u>S19, S20)</u>
denovo254	3.1	0.1	Chloromonas rostafinskii HQ404863.1	Chloromonas rostafinskii HQ404863.1	No blast hit
					(82%, 48% - denovo254 vs. <i>Chloromonas rostafinskii</i> : 88%,
	1				84% - denovo254 vs. Chloromonas miwae LC012762.1, thr

					CBCs [one out of in the most conserved part of the structure] when compared denovo254 and <i>Chloromonas miwae</i> , see Figs S12, S14)
denovo23	0.1	2.2	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo259	2.6	0.2	Uncultured Chlorophyta clone ALBC6 JX435348.1	Uncultured Chlorophyta clone ALBC6 JX435348.1	Uncultured Chlorophyta clone ALBC6 JX435348.1 (100%, 100%)
denovo76	0.1	1.9	Chloromonas alpina CCCryo032-99	Chloromonas alpina CCCryo032-99 HQ404864.1	Chloromonas alpina CCCryo032-99 HQ404864.1 (100%, 100%)
denovo219	1.8	0	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo85	1.5	<0.1	Chloromonas pichinchae CCCryo 2616-06 HQ404889.1	Chloromonas pichinchae CCCryo 2616-06 HQ404889.1	Chloromonas pichinchae CCCryo 2616-06 HQ404889.1 (95%, 100%), see Figs S6, S7
denovo166	0.1	0.8	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo74	0.6	0.3	Raphidonema nivale AJ431676.1	Raphidonema nivale AJ431676.1	uncultured Chlorella sp. 5 AB903011.1 (100%, 100%)
denovo93	0.7	0	No blast hit	No blast hit	Stichococcus sp. SAG 2482 KX094848.1 (100%, 100%)
denovo35	<0.1	0.5	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo69	0.6	0	No blast hit	No blast hit	No blast hit (82%, 52%, <i>Prasiola delicata</i> MF801375.1, six CBCs in helix III, see Figs S21, S22)
denovo36	<0.1	0.5	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo127	0.3	0.1	Chloromonas rostafinskii HQ404863.1	Chloromonas rostafinskii HQ404863.1	No blast hit (85%, 48% - denovo127 vs. <i>Chloromonas rostafinskii</i> : 90%, 82% - denovo127 vs. <i>Chloromonas miwae</i> LC012762.1, two CBCs in helix III [one CBC out of it in the most conserved part of the structure] when compared denovo127 and <i>Chloromonas miwae</i> , see Figs S12, S14)
denovo13	0.3	0	No blast hit	No blast hit	No blast hit (84%, 85%, <i>Desmococcus endolithicus</i> KX094830.1; four CBCs in helix III, see Figs S19 and S20).
denovo44	0.2	0	Uncultured Chlorophyta clone ALBC6 JX435348.1	Scotiella cryophila K-1	<u>Scotiella cryophila K-1</u> (96%, 100%, 4CBCs in helix III [outside the most conserved part] when compared denovo44 and <i>Scotiella cryophila</i> K-1, see Figs S15, S16)
denovo249	0.2	0	Uncultured Chlorophyta clone ALBC6 JX435348.1	Chloromonas brevispina K-2	<u>Chloromonas brevispina K-2</u> (96%, 82%, one CBC in helix III [outside the most conserved part] when compared denovo249 and <i>Chloromonas brevispina</i> K-2, sequence-secondary structure alignment in Fig. S5)
denovo188	<0.1	0.1	No blast hit	No blast hit	No blast hit
denovo101	0.2	<0.1	No blast hit	No blast hit	 (no significant similarity found) No blast hit (84%, 75%, <i>Chloromonas miwae</i> LC012762.1; three CBCs in helix III [two out of in the most conserved part of the

					structure] when compared denovo101 and <i>Chloromonas miwae</i> , see Fig. S13)
denovo168	0.2	0	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo143	0.1	0	No blast hit	No blast hit	Stichococcus sp. KX094857.1 (99%, 100%)
denovo109	0.1	0	No blast hit	No blast hit	<i>Stichococcus</i> sp. KX094848.1 (89%, 89%, four changes at both side of secondary structure found in less conserved part of the structure, likely no CBC, Figs S17, S18)
denovo28	0.1	0	No blast hit	No blast hit	Lobosphaera sp. K-1 KT119889.1 (94.0%, 100%), no CBC found when compared denovo28, Lobosphaera sp. K-1 KT119889.1 and Lobosphaera incisa KM020046.1, so denovo28 can be assigned to Lobosphaera incisa, sequence- structure alignment in Fig. S8
denovo124	0.1	0	No blast hit	No blast hit	<i>Lobosphaera incisa</i> KM020046.1 (94%, 100%), see Figs S8, S9
denovo45	0.1	0	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo72	<0.1	0.1	Chlamydomonas nivalis GU117577.1	Chlamydomonas nivalis GU117577.1	<i>Chlamydomonas nivalis</i> GU117577.1 (94%, 100%), no CBC when denovo72 compared to <i>Chlamydomonas nivalis</i> DL07; [interestingly, ITS2 of denovo72 has higher sequence similarity with Uncultured <i>Chlamydomonas</i> clone H14 KX063729.1 (98%, 100%) but there is one CBC in helix II between denovo72 and clone H14], see Fig. S10, S11.
denovo180	0.1	0	No blast hit	No blast hit	No blast hit (84%, 100%, <i>Chlorocloster engadinensis</i> FM946011.1; one CBC in basal part of helix I and one CBC in the basal part of helix II, see Figs S23, S24)
denovo212	<0.1	0.1	No blast hit	No blast hit	Trebouxia sp. MG098229 (100%, 100%)
denovo173	0.1	0	Chloromonas sp. CCCryo289-06 HQ404893.1	<u>Chloromonas brevispina K-2</u>	<u>Chloromonas brevispina K-2</u> (100%, 75%, two CBCs in helix III [outside the most conserved part] when compared denovo173 and <i>Chloromonas</i> brevispina K-2, sequence structure alignment in Fig. S5)
denovo266	0.1	0	Uncultured Chlorophyta clone ALBC6 JX435348.1	<u>Chloromonas brevispina K-2</u>	<u>Chloromonas brevispina K-2</u> (96%, 69%, 2 CBCs in basal part of helix II and 1 CBC in helix II when compared denovo266 and <i>Chloromonas</i> brevispina K-2, sequence structure alignment in Fig. S5)
denovo248	<0.1	<0.1	Chloromonas sp. CCCryo289-06 HQ404893.1	<u>Chloromonas brevispina K-2</u>	No blast hit (92%, 54%, 6 CBCs when compared denovo248 and <i>Chloromonas brevispina</i> K-2, one CBC out of it is located in the most conserved part of the structure, sequence structure alignment in Fig. S5)
Other	1.2	0.4			